Neurosciences Research Program

Bulletin

Volume II SEPTEMBER-OCTOBER 1964 Number 5

C O N T E N T S

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SPONSORSHIP AND SUPPORT

The Neurosciences Research Program is sponsored by the Massachusetts Institute of Technology. It is supported in part by National Institutes of Health Grant No. GM 10211-03; National Aeronautics and Space Administration Grant No. NsG 462 Amendment 1; Office of Naval Research Grant Nonr(G)-00089-64; The Rogosin Foundation, and Neurosciences Research Foundation, Inc.

SOME BRAIN STRUCTURES AND FUNCTIONS RELATED TO MEMORY

A Summary of an NRP Work Session
held January 10-11, 1964
and of Subsequent Discussion at the
Seventh Stated Meeting of NRP Associates

by

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INTRODUCTION

The NRP Work Session on "Brain Structures and Functions" was held on January 10-11, 1964 at the NRP Center on the Brandegee Estate in Brookline, Massachusetts.

NRP Chairman Francis O. Schmitt explained that the Work Session was designed as part of a program to bring to the Associates, particularly those from the physical and chemical fields, current data and ideas about brain mechanisms for recording, storing, and retrieving information.

In keeping with this program, the Work Session co-chairmen, Drs. Walle Nauta and Sanford Palay, presented a summary of the session at the Seventh Stated Meeting of NRP Associates (February 10-12, 1964). Remarks made by several of the Associates and guests at the Stated Meeting have been incorporated into this text. Those Stated Meeting participants whose comments appear in this report are listed below:

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The Work Session on Brain Structure and Function was organized around two problems: 1. the possibility of identifying within the central nervous system any neural organization that is of more immediate significance than others for the phenomenon of memory; and, 2. evidence for both stability and mutability of innately specified functional connections. In addition, some aspects of the classical riddle of frontal lobe function were discussed, in order to familiarize the non-behavioral group members with some of the problems encountered in animal experiments concerned with the storage and recall of experiences.

I. FOREBRAIN STRUCTURES AND MEMORY

A. The Hippocampal Formation

It is well known, mainly from clinical experience, that even fairly extensive destruction of certain brain regions need not seriously affect the capacity for storage and recall of experiences; while, on the other hand, lesions of similar or even much smaller size localized in other brain regions may cause striking impairments of this capacity. A notable example of the latter category of structures is the hippocampal formation, as demonstrated, among other findings, by Scoville and Milner's observation of apparently enduring impairment of the memory storage function in patients who had undergone partial bilateral removal of the hippocampus (1).

In view of this and other evidence (2), the first part of the Work Session was devoted to a discussion of the structure of the hippocampal formation, and its position in the known anatomical circuitry.

The hippocampus, which can be described as the free medial edge of the cortical hemisphere, is present in all vertebrates. Concomitant with the development of a massive neocortex in mammals, the hippocampal formation becomes "rolled in" to the ventricular lumen to form a characteristic horse-shoe shaped structure (see Fig. 1). Two gyri, the dentate gyrus (gyrus dentatus or fascia dentata) and Ammon's horn, can be distinguished; together with a fiber tract, the fornix (fimbria fornicis), which represents some afferent and apparently most of the efferent connections of the hippocampus (see Figs. 2 and 3).

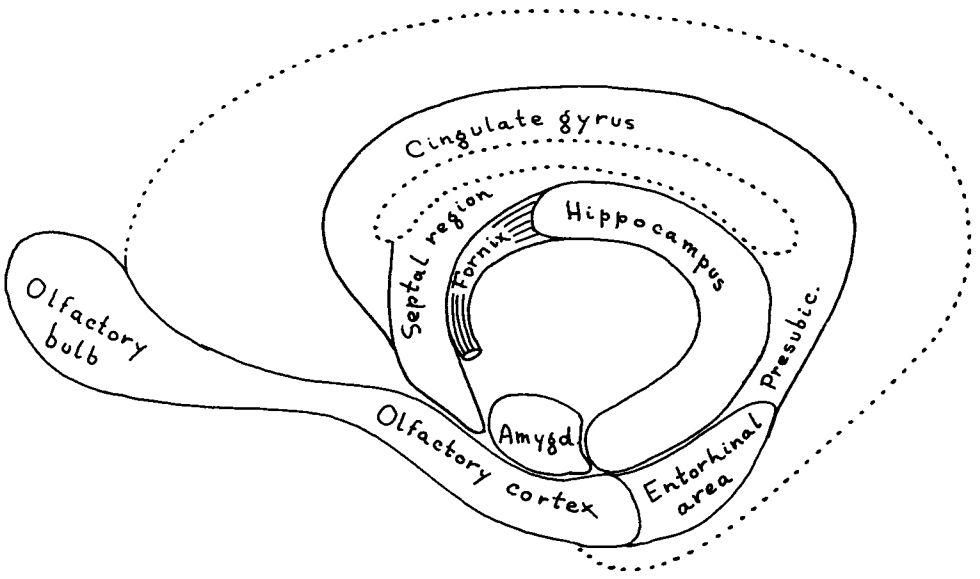


Figure 1. Half-schematic medial view of the right hemisphere of a hypothetical small mammal. The outline of the neocortex is shown in stipple, as is that of the cross-section through the corpus callosum (the massive plate of axons interconnecting the left and right neocortical hemispheres). The various neural structures assembled in the form of a ring around the hilus of the hemisphere were collectively named "the great limbic lobe" by Broca. Owing to the notion that the entire complex represented the sense of smell, the complex later became known as the "rhinencephalon" or "olfactory brain." As these terms were recently found to be unduly restrictive, the complex is now commonly referred to as the limbic system.

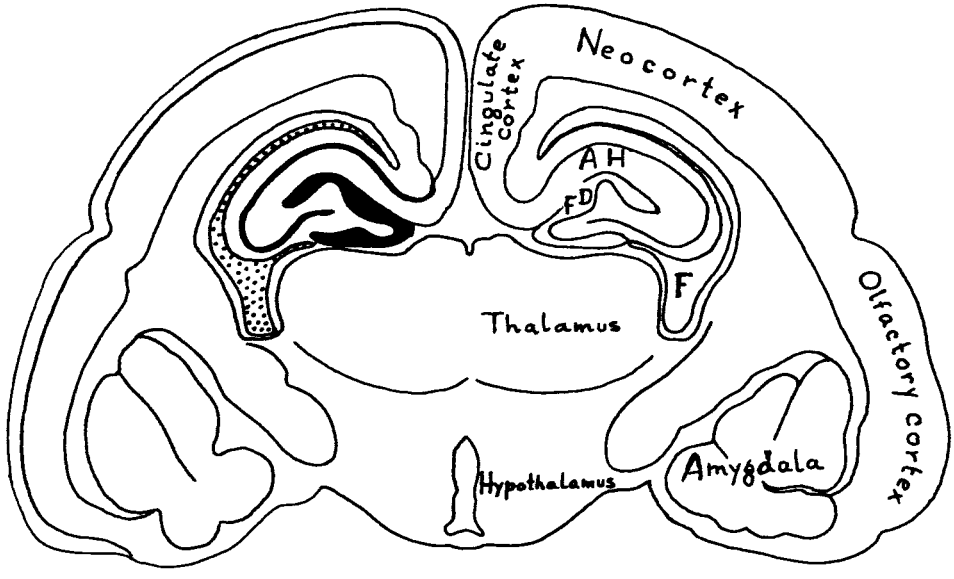


Figure 2. Transverse section through the forebrain of a small mammal (hedgehog). The hippocampal formation appears in the upper half of the section; it is composed of Ammon's horn (AH), the fascia dentata or gyrus dentatus (FD), and the fimbria of the fornix (F). The orientation of the section corresponds to that of Cajal's drawing shown in Figure 3.

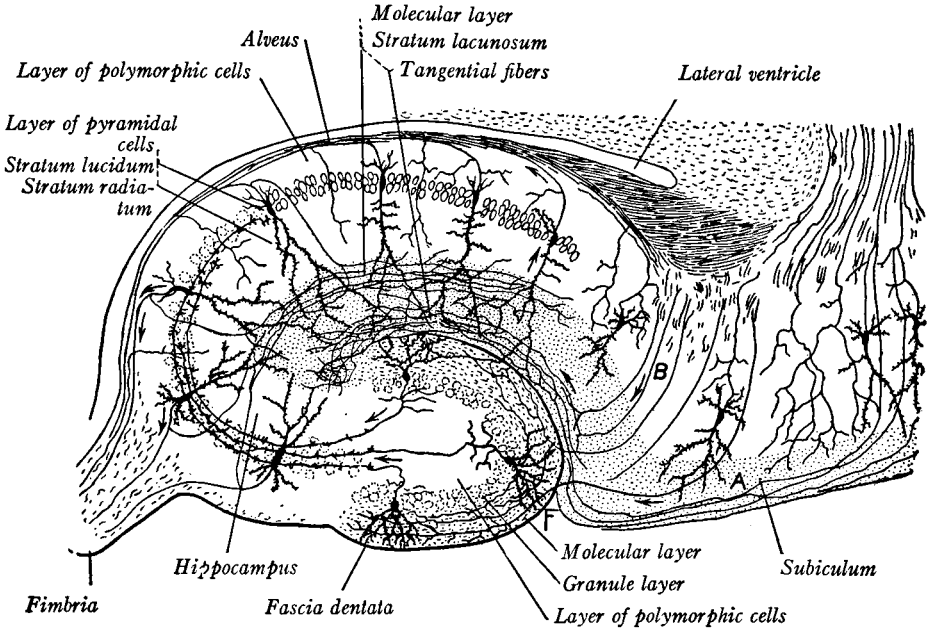


Figure 3. Simplified diagram of the hippocampal formation of the mouse. The arrows show the direction of conduction; A, molecular later, and B, pyramidal cell layer of the subiculum; F, hippocampal fissure. [From Ramón y Cajal.]

1. Histological Structure

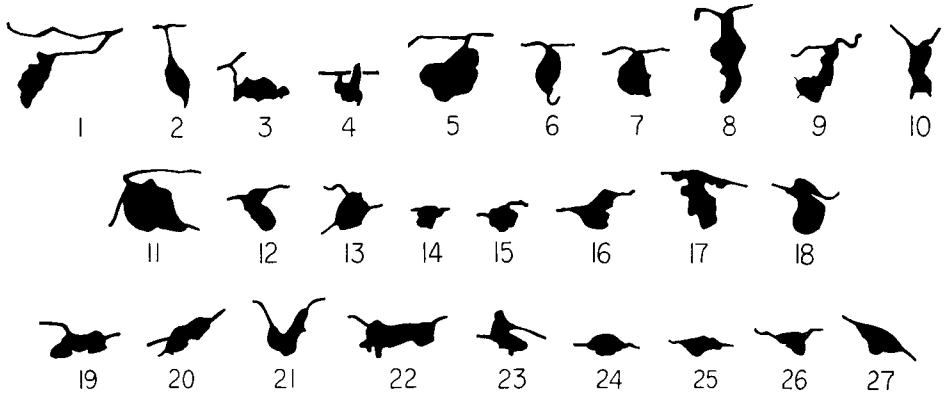
Dr. Jay Angevine gave a detailed and profusely illustrated account of the morphology and interconnections of the hippocampal neurons. Only a few points of his account can be stressed here. The main influx of impulses to the hippocampal formation appears to take place over fiber systems terminating in synaptic contact with the dendrites of the cells of the dentate gyrus. These cells emit extremely thin axons which form complex skeins of so-called "mossy fibers" distributing to the characteristic "double pyramid" cells (Ammon's pyramids) of the Ammon's horn. The synapses of this connection are found mostly on the proximal part of the long apical dendrite; they are highly typical and consist of varicose, bag-like swellings of the mossy fiber engulfing a spinous thorny protrusion of the dendrite stem (see Figs. 4A and 4B). McLardy's histochemical studies have demonstrated high concentrations of zinc ions in the general zone of mossy fiber distribution (3). It is not yet known whether the metal is localized in synaptic structures proper, or in what form the metal occurs. The apical dendrites have many further synaptic relationships with other afferent fibers, notable among which are widely distributed collaterals of the axons arising from other Ammon's pyramids. These collateral connections are quite numerous and represent a widespread system of communication among the Ammon's pyramids, affecting mostly the distal positions of the latter's apical dendrites. The approximate distribution of the various synaptic contacts on the surface of the Ammon's pyramids is shown in Figure 4C.

The main branch of the axons of Ammon's pyramids contributes to the formation of the fornix bundle, which represents the only known efferent connections of the hippocampal formation. (Other outgoing pathways are believed to lead to the temporal lobe cortex, but the anatomical evidence in this category is still inconclusive).

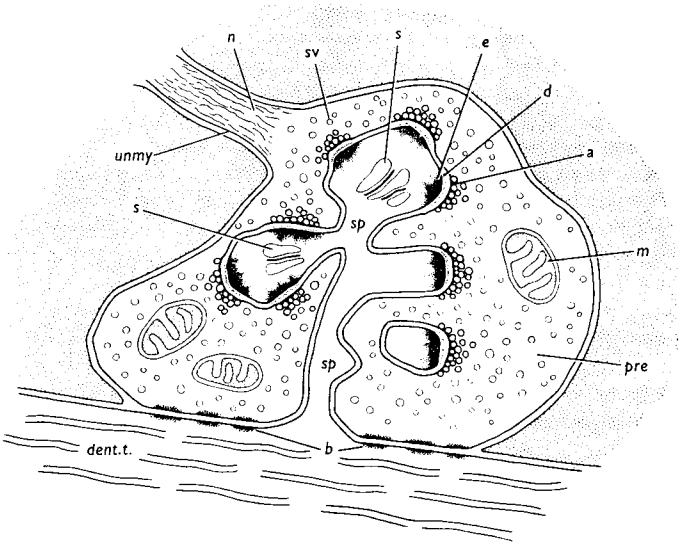
2. The Place of the Limbic System in the Neuronal Organization

The hippocampus forms part of a heterogenous array of neural structures which occupy the medial and basal walls of the hemisphere and are currently subsumed under the term, limbic system. Other prominent representatives of this organization are the amygdaloid complex and the gyrus fornicatus. Together, the limbic structures are arranged in a ring-like

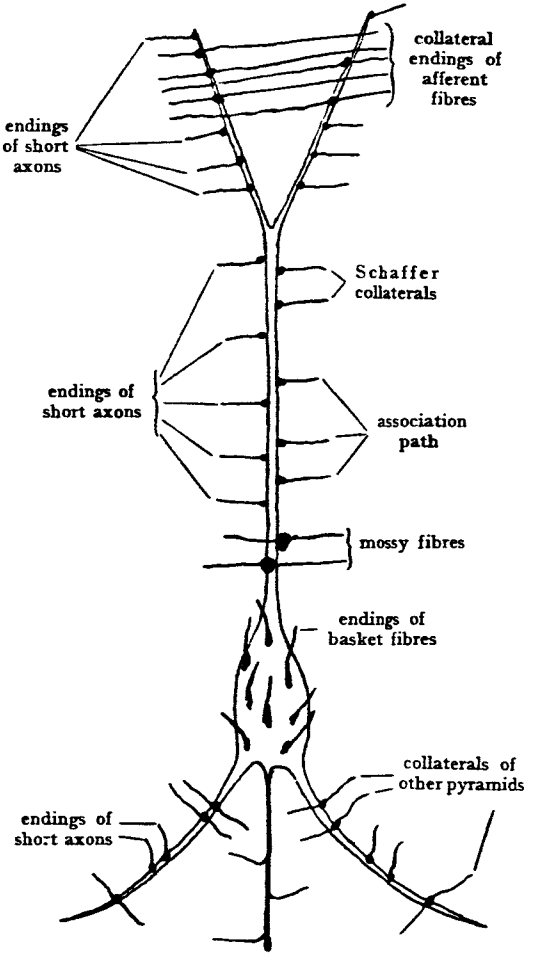
Figure 4. DIAGRAMS OF SYNAPSES UPON HIPPOCAMPAL PYRAMIDAL CELLS.



4A: Bag-like swellings of the mossy fiber; Golgi stain; magnification 1,300X. [Reproduced from page 136 of "Special Axo-Dendritic Synapses in the Hippocampal Cortex: Electron and Light Microscopic Studies on the Layer of Mossy Fibers," by T.W. Blackstad and A. Kjaerheim, in Vol. 17, No. 2 (Oct. 1961) of J. Comp. Neur.]



4B: Fine structure of a mossy fiber ending. [Reproduced from page 115 of "The Fine Structure of the Mossy Fibre Endings in the Hippocampus of the Rabbit," by L.H. Hamlyn, in Vol. 96, Part 1 (Jan. 1962) of J. of Anat.]



4C: Origin of synapses and their relative distribution. [Reproduced from page 168 of "Studies on the Structure of the Cerebral Cortex; II: Continuation of the Study of the Ammonic System," by Lorente de Nó, in Band 36, Heft 2 und 3 (1934) of J. für Psychologie und Neurologie.]

manner around the hilus of the cerebral hemisphere (Fig. 1). Originally conceived in its totality as the olfactory brain ("rhinencephalon"), the limbic system is now believed to be involved in functions far less specific than mere olfaction -- functions related to attention-focussing and, more generally, to "behavioral attitude" reflecting vital feelings and affects. It is nevertheless certain that olfactory impulses have access to the limbic system; in fact, the olfactory cortex, which receives impulses from the olfactory bulb via the olfactory tract, is located in the rostral part of the temporal limb of the gyrus fornicatus. In man it corresponds to the uncus of the temporal lobe. However, it now appears certain that the activity of the hippocampal formation is also affected by afferent impulses representing modalities other than the olfactory sense.

Little is known about the anatomical pathways conveying sensory information to the hippocampus. It appears certain that the structure has no specific sensory afferent pathways comparable to those distributing to the thalamo-neocortical organization. The main neural influx, as far as can be judged from current anatomical data, reaches the hippocampal formation from two directions: 1. from the brain-stem reticular formation, apparently mostly via the septal region; and, in apparently greatest volume, 2. from the gyrus fornicatus, in particular from two of the latter's subdivisions bordering on the hippocampus: the presubiculum and the entorhinal area. The pathways from the reticular formation cannot be identified in terms of any sensory modality, and appear to represent a multimodal ("non-specific") conduction system. The same appears true of the afferents from the gyrus fornicatus: the latter's associations are highly complex and include afferents from at least frontal and temporo-occipital areas of the cerebral cortex, both of which are classified as "association areas," i.e., regions not identifiable as subserving any particular sensory system. In summary, it appears that the hippocampus is the recipient of several forms of "amalgamated" information, coming in part from the subcortical levels, in part also from the cerebral cortex.

The efferents from the hippocampal formation are equally complex and difficult to characterize in easily interpretable neurological terms. The main outflow system of the hippocampus is the fornix (see page 5) composed largely of axons from the Ammon's pyramids, and receiving further contributions from the gyrus fornicatus. The fornix and its

trans-synaptic extensions in large part reciprocate the afferent conduction system ascending to the hippocampus from the mesencephalic reticular formation. Both the ascending and descending limbs of this reciprocating, circuitous connection make numerous synaptic contacts in the hypothalamus. This diencephalic subdivision of the reticular formation has been long known to encompass important neural regulating mechanisms subserving visceral and endocrine functions, including thermoregulation, the diurnal rhythm, osmoregulation, etc. More recently, using electrical stimulation through indwelling electrodes in relatively free-ranging animals, Olds and Milner have obtained evidence suggesting that the activation of certain neural mechanisms in the hypothalamus is experienced by the animal as "pleasurable" ("reinforcing" in psychological terminology), whereas stimulation of sometimes closely adjacent loci may lead to aversive experiences (4). A more specific recent finding is that of similarly located mechanisms apparently subserving the experiences of hunger vs. satiation (5).

In summary, it appears that the hippocampus, like probably all of the limbic forebrain structures, entertains reciprocal relationships with the mesencephalic reticular formation. The functional state of the hypothalamus, a nodal intermediary in this interrelation, appears likely to be continuously and profoundly influenced by the neural events taking place in the larger hippocampo-mesencephalic circuit. This larger circuit is accessible to sensory information which has already been processed by several analyzer systems: impulses signalling events in the visceral sphere and all those sensed as pain, for instance, appear to reach it via various more caudal levels of the brain-stem reticular formation. (These levels are characterized by an apparent abundance of neurons showing convergence of afferents, i.e., receiving impulses pertaining to more than one sensory modality.) In mammals, further afferents to the circuit arise from the neocortex; the areas of origin of such afferents likewise are characterized, even though at a much higher level of neural complexity, by being gathering places for multiple forms of information concerning the organism's environments.

An intensive discussion developed around the question as to how a neural mechanism such as that composed of the hippocampus and its associations with the brain-stem reticular formation could play an apparently decisive role in the memory process. First, in regard to the internal structure of the

hippocampal formation, Dr. Livingston remarked that especially in the case of the hippocampus itself, it could be doubted that synaptic inter-relations form the only basis for inter-neuronal transmission; the complexity and spacing of tissue elements in the structure would seem to present favorable conditions for ephaptic (i.e., non-synaptic) interactions based on electrical field forces (6). However, Dr. Palay remarked that such "cross-talk" between neurons could be meaningful only if certain time requirements were met; he doubted that this could be the case. He also stressed that the low resistance of intracellular material could form an insulator against ephaptic cross-talk. Dr. Nauta suggested that the mossy fiber system with its large bag-like synaptic contacts and its extremely thin fiber calibre might hold an important clue as to hippocampal function; in fact McLardy has speculated that the system might serve as a mechanism for delay (i.e., temporal dispersion) in the impulse conduction (7).

Dr. Jasper emphasized his experience that memory defects even more severe than those found in cases of hippocampal damage result from apparently small lesions in the hypothalamo-mesencephalic transition area. He specifically recalled a patient with pathology in this region who was permanently unable to store any memory of events occurring after his illness. This localization is the classical one long associated with Korsakoff's syndrome. Dr. Nauta remarked that an alternative (or supplementary?) region of pathology in cases of Korsakoff's disorder, recently identified by Victor, corresponds to the medial component of the mediodorsal thalamic nucleus (8). Both the mesodiencephalic region and the thalamic region indicated by Victor's findings are known to receive projections from the limbic system. Withal, most of the neuropathological findings in cases of memory loss appear to point to some special significance of the limbic system in the memory phenomenon (9).

Dr. Schmitt asked if the available evidence suggests that the hippocampus actually functions as the <u>store</u> of past experiences. Dr. Jasper answered that this appears not to be the case; in fact <u>it is disproved by the finding that the amnesia resulting from bilateral hippocampal lesions does not greatly affect the memories recorded before the surgery</u> (10). The most striking deficit seems to be in the recording of events experienced by the patient after his operation.

B. Elicitation of Memories by Electrical Stimulation of Temporal and Occipital Cortex

At this junction, Dr. Jasper presented the results of a recent re-study by Penfield and Perot (11) of the observations previously described by him and Dr. Penfield in patients treated surgically for temporal lobe epilepsy. In 50 of a total of 440 patients in whom the exposed occipito-temporal cortex was systematically explored, electrical stimulation of certain cortical loci elicited a recall of events -- visual or auditory depending on the locus stimulated -- more vivid and detailed than is normal in spontaneous recall. Examples of visual experiences elicited by such stimulation are listed in Fig. 5 reproduced from Penfield and Perot (1963). The episodes have a normal temporal order and could be likened to a sequence of tape-recordings being played back. They do not outlast the duration of the stimulus, but a sequence interrupted by the cessation of the stimulus often is resumed at the stop point, when stimulation is again applied. In 40 per cent of the cases, the recall episode was followed by a seizure with ensuing amnesia for the experience. Stimulation in the region of the hippocampus and amygdala caused amnesia in about the same percentage of cases, but only if the intense after-discharge set up by the stimulus in the two limbic structures was suddenly propagated to the overlying temporal neocortex. Since recall episodes are discrete, and their content orderly and complete, it seems preferable to speak of the storage of memories rather than memory. Jasper and Penfield do not interpret their observations as proof of storage of the elicited memories in the explored cortical regions, per se. They cautiously leave the possibility open that much more widespread neural mechanisms are involved in the storage, even though "triggering" can take place from the cortical surface.

C. Frontal Lobe Function and Memory

Following Dr. Jasper's presentation, Dr. Haldor Rosvold gave an account entitled "Some physiological studies of frontal lobe deficits in relation to memory function." Dr. Rosvold stressed the difficulty in identifying "loss of memory" in clinical studies and animal experiments. In humans subjected to therapeutic electroconvulsive treatment, for example, the most commonly used clinical test requires the patient to repeat a series of digits after an interval during which another series or sequence of nonsense syllables is interjected.

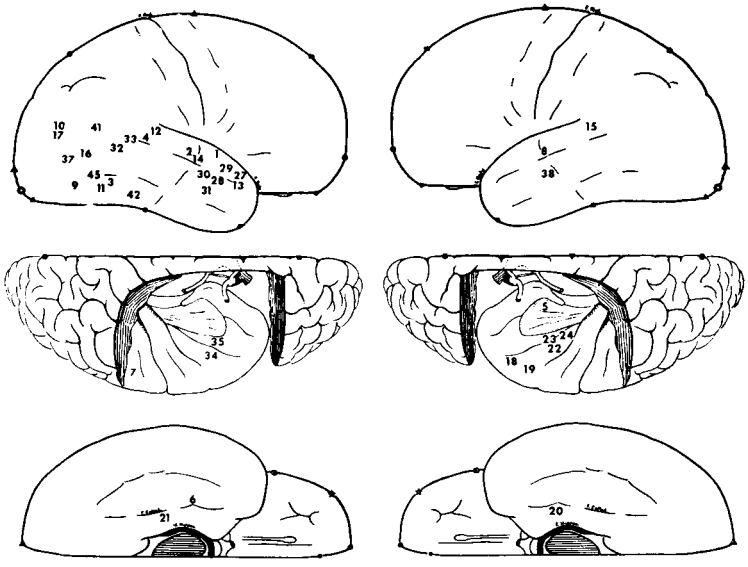


Figure 5. Visual experiential responses to stimulation:

1. familiar street scene
2. a person
3. a person
4. an object
5. familiar scene
6. a face in a picture
7. people
8. familiar man grabbing a stick
9. a friend
10. familiar machine
11. familiar nurses
12. familiar scene
13. people
14. a scene
15. a man fighting
16. a woman
17. steps with people on them
18. scene with people
19. a scene
20. a scene
21. familiar room
22. people
23. a man
24. a man smoking
27. a scene
28. a person
29. people and her mother in the living room
30. a person
31. an object
32. a man
33. familiar man
34. an object
35. his mother at home
37. familiar menacing man
38. herself in childbirth
41. robbers with guns
42. robbers
45. his brother at home in their yard.

[Reproduced from page 671 of "The Brain's Record of Auditory and Visual Experience -- A Final Summary and Discussion," by W. Penfield and P. Perot, in Vol. 86, Part IV (1963) of Brain.]

Patients who fail on this test show considerably better recall when the series first presented consists of familiar, meaningful words.

Recording of memory is clearly related to the processes of association. The literature on the use of electroconvulsive shock (ECS) to disrupt immediate memory in rats was surveyed. Dr. Rosvold emphasized that there is considerable disagreement in the field at the present time, and that it would be premature to accept without qualification the finding that electroconvulsive therapy disrupts immediate memory.

Dr. Rosvold described his experiments using monkeys subjected to bilateral lesions of the frontal lobe. Such animals exhibit a striking deficit in performance when given "delayed response tests." In essence, these tests are arranged as follows: the animal is shown under which one of two identical covers a peanut is placed. A screen is then lowered which prevents the monkey from responding until the screen is raised several minutes later, when the animal is required to remember the correct position of the reward. In a variant test (delayed alternation), the peanut is placed with regular alternation under the right and left covers, but since the animal is not shown the placement, it must remember under which cover it last found the peanut and choose the opposite. Normal monkeys learn to achieve high scores on both tests, but their performance is severely and permanently impaired following bilateral frontal lesions. Although at first glance this impairment could be interpreted as an "uncomplicated" loss of memory function, the results of other functional tests do not support this conclusion (12). It is now apparent that the ineffectiveness of the operated animals is due to an ill-defined change in responsiveness to the environment, the central feature of which has been formulated variously as "incapacity to change set," "perseveration of response," or "stimulus binding." (The latter term refers more specifically to the tendency of animals with frontal lobe defects to over-respond to normal cues as if unable to suppress responses to non-essential stimuli.) Dr. Jasper remarked that learning could indeed be interpreted as "learning what <u>not</u> to do": i.e., if one is taught to take the Northside bus one actually learns not to get on any other bus.

The emphasis of Dr. Rosvold's presentation was that the testing of memory function is a complicated procedure subject to many misinterpretations. Therefore great care

must be exercised in designing experiments in which "memory" is the dependent variable.

Dr. Rosvold pointed out that although the typical frontal lobe syndrome appears in cases of complete frontal lobectomy, it also appears following the removal of the relatively restricted part of the frontal cortex lining the sulcus principalis. Although unattainable by any cortical lesion outside the frontal lobe, similar behavioral deficits appear in cases of certain subcortical lesions, involving namely the head of the caudate nucleus, or the rostral part of the hippocampus. The following table summarizes the effects of various surgical lesions upon, respectively, visual discrimination and delayed response ability:

Removal of:	Discrimination + □	Delayed Response
Frontal Lobe	Normal	Absent
Infratemporal Cortex	Absent	Normal
Parietal Cortex	Absent (visual)	"
Amygdala	Normal (tactile)	"
Caudate	"	Absent
Hippocampus	"	"
Cingulate	"	Normal

Somewhat surprisingly, lesions in the dorsomedial thalamic nucleus, a major source of impulses to the frontal cortex, fail to produce the functional loss. Another striking finding is that frontal cortex removal extensive enough to cause functional impairment if performed in the adult monkey, does not interfere with the development of a normal delayed response capacity when performed in very young monkeys. (They may be one or two weeks old -- the duration of this permissive period has not been established.) This observation once again raises the question of functional plasticity of immature brains: it is as if at such early stages the central nervous system has a reserve of "uncommitted" neural organization large enough to permit compensation for apparently massive loss of neurons. In this connection, Dr. Livingston recalled the observation that unilateral resection of the visual cortical area 17 in the newborn kitten does not result in behavioral evidence of impaired vision. Dr. Teuber, however, remarked that it seemed doubtful in these experiments that the entire cortical projection of the lateral geniculate

body had been resected.

D. Discussion

The general conclusions drawn from the preceding presentations were that 1) the "memory storage mechanism" cannot be identified at this time, and, 2) the limbic system, and more especially the hippocampus, although clearly of importance in the storage of memory, cannot itself be the "storehouse." Discussion was then directed towards other possible roles of the hippocampus in memory. Dr. Schmitt remarked that the hippocampal function might be comparable to that of a valve or amplifier. After lively group discussion, Dr. Livingston suggested that the hippocampus appears to contain a mechanism capable of emitting a signal amounting to a "Now print!" message, without which no recording can take place. This "Print!" message could be related to "affective color" or "emotional tone." The significance of affective color for storage and recall of experiences has long been recognized, and evidence exists linking the hippocampus as part of the limbic system to the functions of affect, behavioral attitude, and attention focussing. Drs. Kety and Weiss both objected that evidence of memory has been demonstrated even in the spinal cord, and that it appeared possible to postulate that any neural organization is endowed with this capacity. Dr. Livingston replied that "spinal cord memory" requires a much greater repetition of the input sequence than is needed for most learning processes at the forebrain level; it seemed conceivable to him that the limbic system is essentially an accelerator mechanism for such behavioral memories. It is undoubtedly true that certain behavioral responses can be and are learned by intact animals after a single trial, and no similar fast conditioning has been observed in the spinal cord. Dr. Galambos drew attention to the unequal susceptibility of simultaneously learned behaviors to electroconvulsive shock treatment (ECS): avoidance behavior conditioned by the pairing of a neutral conditioned stimulus with a noxious unconditional stimulus can be abolished by ECS much more readily than can forms of behavior conditioned by reward (e.g., lever pressing for food). Dr. Jasper remarked that this same dissociation has been obtained by the use of chlorpromazine instead of ECS. Dr. Kety interpreted this as an indication that memories are coded (perhaps chemically) on the basis of their "affective color," and their recall is typically accompanied by an affect corresponding to the original experience. He inquired if this did not, therefore, indicate that the

hippocampus is involved in the circuitry of recall as well as that of storage. Replying negatively, Dr. Jasper noted that those memories that can be activated by patients with hippocampal defects appear to have the appropriate affective colorings. Dr. Nauta suggested that the hippocampus, although apparently essential for the consolidation of memories, need not be directly involved itself in the recall mechanism if the affective concomitants of recall were "stored" in subcortical regions or, alternatively, in components of the limbic system other than the hippocampus. Dr. Bullock remarked that the trend of all our evidence made it increasingly hard to consider memory to be a unitary mechanism; in fact it seems to indicate a complex of essentially different phenomena. Dr. Schmitt countered that the reductionist approach, which has permitted geneticists to discover a basic mechanism underlying apparent diversity, may resolve the memory problem in the same manner.

II. INNATE, RELATIVELY STABLE NEURAL ORGANIZATIONS: THE PROBLEM OF MUTABILITY OF EXISTING FUNCTIONAL CONNECTIONS BETWEEN NEURONS

Dr. David Hubel gave the next lecture, "The neuronal organization of the visual system in the cat and monkey," a comprehensive overview of his recent detailed neurophysiological studies with Wiesel. They have sought to analyze the manner in which visual stimuli impinging on the retina are processed by the various neural processing stations in the visual pathway. The main technique used in these studies consists of microelectrode recordings from single neurons of the retina, lateral geniculate body and visual cortex, when such neurons are activated or inhibited by photic stimuli of various physical parameters (diffuse vs. local, static vs. moving, geometrical shape of the stimulus, spectral composition, etc.). The wealth of well-integrated data presented in Dr. Hubel's account will be greatly condensed here.

As shown in Fig. 6, the three major components of the visual apparatus studied in Hubel and Wiesel's experiments are: 1. the retinal ganglion cells, which receive impulses from the photoreceptor elements (rod- and cone-cells) via the so-called bipolar cells (impulses arriving at the retinal ganglion cells have thus already passed a minimum of two synaptic junctions); 2. the lateral geniculate body, which receives impulses directly from the retinal ganglion cells; and 3. the visual cortex, which receives the geniculo-cortical

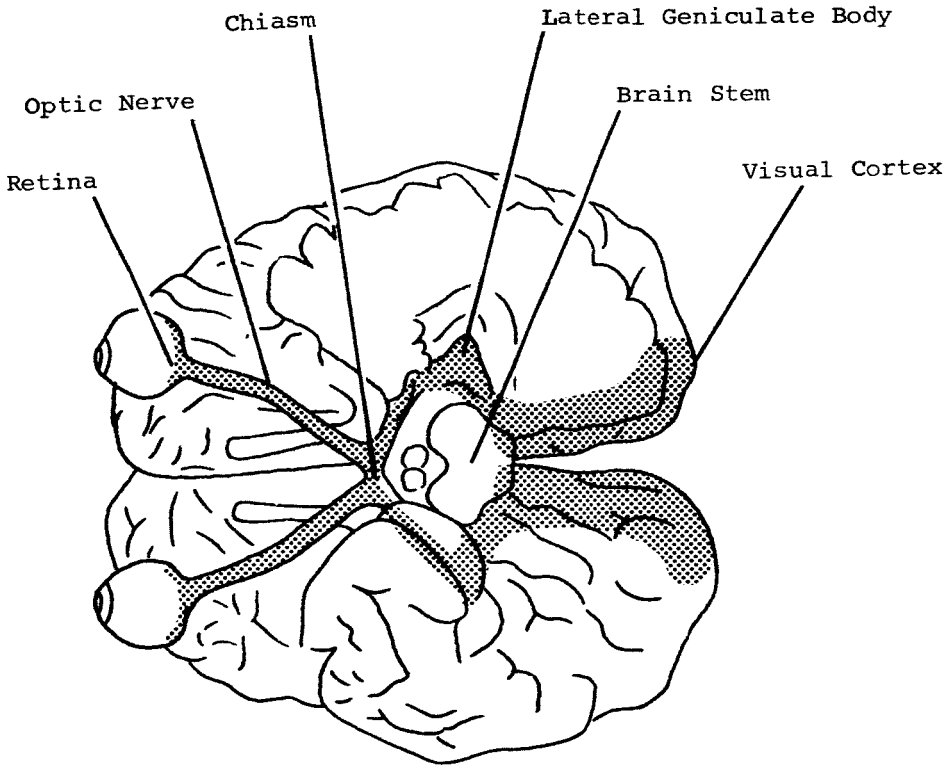


Figure 6. Visual system appears in this representation of the human brain as viewed from below. Visual pathway from retinas to cortex via the lateral geniculate body is shown in stipple. [Adapted from p. 55 of "The Visual Cortex of the Brain," by D. H. Hubel, in the November 1963 issue of Scientific American.]

projection.

As a rule, diffuse illumination of the retina causes less change in the existing spontaneous activity of neurons at successively higher levels in the pathway, and little or no change at the level of the striate cortex. Generally, neurons of the visual system respond maximally only when stimuli of particular physical characteristics are applied to the retina.

A. Retinal Ganglion Cells

It appears that ganglion cells of the retina have roughly circular receptive fields. For one category of ganglion cells ("on" cells) the receptive field consists of an excitatory central area surrounded by an inhibitory ring, while the field for "off" cells is characterized by an inhibitory center with an excitatory surround. The relative ineffectuality of diffuse retinal illumination is probably due to mutual cancellation of excitatory and inhibitory effects. It appears likely that the information transmitted by the retinal ganglion cells to the lateral geniculate body is coded largely in terms of movement, border and contrast, and does not emphasize the absolute level of illumination. As to color perception, some ganglion cells in the monkey, but none in the cat, have been found to respond selectively to colored light.

B. Lateral Geniculate Body

Cells of this way-station between the retina and the visual cortex show characteristics comparable to those of the retinal ganglion cells. As in the retina, "on" and "off" cells could be identified, but the antagonism between center and periphery of the effective retinal stimulus area was found to be in general stronger than it is for retinal ganglion cells. These differences suggest that each cell in the lateral geniculate body receives its impulses from more than one retinal ganglion cell.

C. Visual Cortex

Hubel and Wiesel's findings have shown that all cells of the visual cortex can be made to respond to retinal illumination of diverse physical characteristics. In the cat, no cells were found to respond to diffuse retinal illumination, suggesting that "on" and "off" responses cancel precisely, and

that the cat's visual cortex is not designed to register changes in total illumination. The most outstanding characteristic of cells in the visual cortex of both cat and monkey is that most respond maximally to linear (slits, bars or edges) instead of concentric illumination patterns. For most cells, only very specific stimulus characteristics are effective: most often, a bar of light that is moved at a certain tilt across the boundaries between antagonistic areas, and usually in a particular direction, is required to elicit maximal responses. Hubel and Wiesel's studies have shown a topological arrangement of those cells that respond optimally to a specific orientation of a stimulus applied to a particular small area of retina; such cells are arranged in a continuous column extending throughout the thickness of the cortex. The visual cortex can thus be thought of as a mosaic of prismatic columns, each of which functions as a maximal sensor of at least one particular kinetic property of photic stimuli.

D. Binocular Interaction

In most mammals the visual pathway is arranged so that impulses from both eyes are transmitted to each visual cortical area. Although the contralateral input is quantitatively dominant in most vertebrates, in primates the proportion is believed to be about equal. In the cat, 84 per cent of the cortical cells recorded from, could be driven from either the left or the right eye, and about half were found to respond almost equally well to stimulation of either eye, provided that the stimulation were applied to geometrically homologous loci of both retinae and were in either case given identical characteristics of shape, size, and movement. Some cells, however, respond only to simultaneous and homologous stimulation of both eyes.

E. Postnatal Development of the Visual System

Hubel and Wiesel's findings suggest that the organization of the visual system described above is innate: the analysis of retinal, geniculate, and cortical levels of the system in kittens 8 days old (eyes just opened), 16 days old, and 19 days old, gave results comparable in almost all respects to those obtained in the adult cat.

F. Deprivation Experiments

This chapter of Hubel and Wiesel's experiments has furnished some intriguing data regarding the functional

stability of the visual system. In these experiments, performed in cats ranging in age from 8-days to adulthood, one or both eyes were either completely occluded by eyelid suture, or allowed to receive only diffuse light through a milky occluding lens. The results of these experiments indicate that, despite interference with vision in one or even both eyes, the visual system develops normally during the first 19 days of postnatal life. If, however, very young kittens with little or no visual experience are visually deprived in one eye for two to three months, the animals behave afterwards as if their deprived eye were blind. The cell layers of the lateral geniculate body which normally receive their input from the occluded eye fail to grow to normal size. Remarkably, however, both these undersized cells and the retinal ganglion cells nonetheless show normal physiological responses to photic stimulation. In the visual cortex, by contrast, although no cell stunting is apparent, almost no cortical cells can be found to respond to photic stimulation of the deprived eye, whereas about 90 per cent of the cells can now be driven from the normal eye. The conclusions appear inescapable that: 1. photic deprivation of one eye in young kittens causes a functional degradation of pre-existing connections between the geniculate cells related to that eye, and the visual cortex; 2. under such conditions, the intact eye becomes the dominant driving force for all cells in the visual cortex, suggesting the development of new functional connections in the geniculocortical pathway; and 3. plasticity of connections adequate to permit such reorganization in the visual system diminishes progressively with age, and disappears entirely at one year or even earlier.*

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* Hubel: The main conclusions to be drawn from our work as applies to theories of memory or learning are the following: 1) Parts of the CNS that have been examined in sufficient detail for any conclusions to be drawn, suggest a high degree of specificity of neural connections. In the parts of the visual system we have studied, this specificity appears to be innate. There seems to be inadequate evidence at present to permit the conclusion that at progressively higher levels in the nervous system the relation between input and response is progressively less rigidly specified, attractive as this idea may seem intuitively. 2) Deprivation experiments suggest that weakening or disruption of connections can occur if sensory input is distorted, at least at an early age. Some strengthening of connections may also occur, but this is far less clear. Our findings suggest a disorganization, rather than a reorganization [footnote continued on following page]

III. GENERAL DISCUSSION

The foregoing accounts led to a general consideration of anatomical and chemical mechanisms which may determine storage and retrieval of information.

A. Circuit Specificity

Dr. Palay emphasized the combination of complexity and specificity in the neural net. Functioning of any given cell is largely dependent both on afferents quite specifically organized in terms of excitatory or inhibitory effects, and on integration in space, (i.e., exact site of synaptic contact on the cell surface), time, and source or origin. Any molecular theory concerning the workings of the nervous system must take these factors into consideration. Dr. Palay, in a discussion with Dr. Schmitt, allowed that the most rigidly defined relationships between inputs and responses are found in those brain areas immediately involved in the receipt of sensory messages (see Dr. Hubel's account), whereas the farther one goes into the nervous system beyond the limits of such regions, the less rigidly specified the relationship between input and response appears to become. Dr. Kety raised the question whether the circuitry is really immutable: could not individual synapses change or even disappear and be replaced by new synaptic contacts? Dr. Palay objected that this would be incompatible with long-term memory. Dr. Jasper, however, remarked that Dr. Hubel's findings in the visual cortex suggested that even in that well-specified area, the circuit's rigidity is not immutable: for example, when one eye is obstructed shortly after birth, cells normally activated by the obstructed eye become influenced by the opposite eye. Dr. Livingston suggested, as one of the essential features of the nervous system, the presence of highly specified neural organizations, coupled with and partially manipulated by neuronal mechanisms capable of rather radical plastic reorganizations. Dr. Schmitt raised the question of whether recall could be achieved only by activation of the very same neurons that were involved in the original experience, or, conversely, by activity elicited in another neural mechanism that has become equivalent to it. The concensus was in

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[footnote continued from previous page] of neural connections following deprivation. 3) Specificity of information is contained in the neural interconnections and their organization rather than in the frequency of impulses, or their detailed temporal patterning.

favor of the latter supposition.

B. Molecular Specificity

The discussion next moved to the possible nature of the "Now print!" signal. Dr. Schmitt inquired whether a hormone or some other chemical transmitter might be the carrier of such information leading to storage. He stressed the need for data regarding the read-out mechanism, a stage in the information process almost totally obscure, even in genetics, and in all likelihood a decisive step in the process of information retrieval in the brain. Dr. Weiss stated that the obvious question was whether memory can be conceived of as being stored in a mosaic of multiple logic units, and suggested that empirical data seem to answer this question negatively. Dr. Schmitt suggested the possibility that certain neurons become functionally interrelated by virtue of some common specific property of the molecular componentry, a specificity somewhat comparable to that encountered in immunology. He pointed out that the present dilemma lies in the reconciliation of such specificities with the multiplicity of connections present in neural networks. Dr. Palay demurred that it seemed premature to attempt to arrive at molecular theories concerning memory before the phenomena of memory are completely defined. Dr. Schmitt, in reply, cited the considerable progress of genetics, achieved despite a conspicuous lack of information concerning one of the essential steps in the process, namely, read-out. He cautioned against the risk of becoming overly engrossed in complexities, and suggested that the use of molecular-biophysical methods could be fruitful even at the present stage.

Dr. Livingston queried whether it was really necessary to think of "read-out" in the same sense as applies to the genetic process. He outlined a concept of genetically determined but chemotropically effectuated migratory movements of developing neurons away from their matrix layer, followed by similarly determined sprouting of cell processes leading to the completion of a species-specific interneuronal organization. Dr. Weiss remarked that Dr. Livingston's notions of the developmental mechanisms involved in the establishment of circuitry needed some further comment, since it is now clear that the length over which a fiber will grow is not predetermined; only the cell's ability to respond to the environment is genetically coded. Dr. Livingston's process would require intricate articulation between cell nuclear content, cell body

content, and the establishment of the final circuitry. Then, once exposed to the multiple inputs from the environment, neurons would be subjected to various intensities of extrinsic activation involving membrane phenomena (13). It is an attractive premise that the effects of such membrane phenomena are similarly expressed in molecular changes within the cell which in turn affect the circuitry, either by the establishment of new intercellular contacts or by a modification of the existing ones. This notion, in short, proposes continuous experiential modifications of pre-existing species-specific circuitry. Dr. Livingston suggested that, once established, such modifications would lead to modified performance of the net; so that read-out would no longer require the kind of molecular reorganizing interventions within the cell which resulted from initial environmental impingement, but instead would require only the utilization of existing circuitry.

Dr. Eigen objected that the foregoing notions tend to imply that, whereas storage is a molecular process, read-out is not. In fact, however, there can be little doubt that the events constituting signal transmission are of a molecular nature. Read-out is a process in which electricity is directed into one structure rather than another; it could even be called a "switch."

Dr. Eigen expressed approval of the time factors in Dr. Livingston's suggested mechanism: complete protein molecules can be synthesized in 20 seconds; on the other hand, read-out must require much shorter times, so short that no real chemical reactions such as the making or breaking of covalent bonds could be involved. In a more general consideration, Dr. Eigen wondered whether indeed the functions of the brain could be explained by any simple molecular mechanism. That a comparatively simple molecular principle has been found to obtain in genetics does not mean that something similar must be identifiable as the basic principle of brain function. Genetic principles operate at the very beginning of life, as a step between living and non-living matter. How do we know that there may not appear, farther along the developmental line, principles that are no longer molecular in nature? If we are going to devise a molecular theory, then the most sensible approach might be to start with something of known biological importance. Since the number of chemical mechanisms possibly involved is so great, a haphazard start would very likely lead to frustration. Of the likely mechanisms, one is the genetic cycle, another the antibody-antigen interaction, and a third, the transformation of highly structured matter

into some chemical state. This means that we should look at membrane phenomena and enzyme action in the membrane.*

The essential question to be asked, and to be studied with the aid of models, is: what modifications of the purely genetic type of information storage and retrieval are necessary, in order to model something that might resemble a process taking place in the brain? Dr. Palay responded that search should be centered around the problem of how cells interact. Dr. Eigen wondered if this would not already be too complex: returning to the example of genetics, progress was made only after it had become established that the nucleic acids were the carriers of the essential information. This finding resulted from the search for a replicating principle, a search in which little heed was paid to the details of genetic phenomena. Dr. Palay remarked that this search had still not solved either the mechanism or the complexity of genetics. Dr. Eigen replied that it was, of course, clear that the discovery of a central principle could not suddenly explain everything, but that it would greatly aid the search for new approaches to the remaining problems.

Dr. Schmitt remarked that the history of important biological discoveries shows that one has to work from both directions; neither the molecular biophysicists nor the "systems" neurobiologists can say to each other: "You are not dealing with reality." In the case of muscle, for example, it is known that two cellular proteins are essential for contraction: myosin and actin. Is it so unreasonable, therefore, to suppose that an important clue to the function of neurons may be found in a neural protein? Proteins can be isolated in any amount, and many are active antigens. Dr. Eugene Roberts added to this that there is at least one enzyme which is found only in the central nervous system; namely, glutamic acid decarboxylase. Specificities, therefore, may not be confined to proteins. In the same context, Dr. Klüver wondered if the high zinc content of the hippocampal mossy fiber system might not suggest some molecular specificity of a very simple order. Dr. Kety remarked that at the present stage, those accustomed to think in terms of circuits have become quite aware of the probable importance of molecular processes for the construction, dissolution, and modification of circuits. Conversely, molecular biologists have

*EDITOR'S NOTE: Dr. Eigen has since chaired two Work Sessions on these analagous mechanisms, which will soon be reported in the NRP Bulletin.

come to realize that central nervous function depends heavily on circuitry. The important issue can be phrased by the question: "Is the specificity of memory inherent in the specificity of intracellular macromolecules or in the specificity of circuits?" Dr. Weiss raised the question whether in the indeterminate number of circuits available, specific modifications by a chemical selectivity would determine the consonance necessary for meaningful transmission patterns. He also stressed that the foregoing discussion had not taken into account the microenvironment of the cell. A case in point is furnished by hormones circulating in the blood and taken up selectively by certain neurons whose functional state is thereby profoundly modified. The possibility must also be considered that a cell, once having undergone modifications in its molecular mechanism as a result of changes in its componentry, might release into its microenvironment some principle distinctive of that cell. In turn, such changes in its microenvironment might determine changes in the transmission of impulses impinging on the cell. Certainly the interaction between neurons and their chemical milieu deserves as much consideration as the intracellular componentry.

IV. SUMMARY

Morphologic, physiologic, and clinical evidence points to the limbic system, particularly the hippocampal formation, as playing a special role in the memory phenomenon. Although the hippocampus does not appear to be the store of memories, it perhaps acts as a valve, determining which memories are stored.

In view of the plasticity of the nervous system, even in brain areas as relatively rigidly defined as the visual cortex, a model of the anatomical and chemical mechanisms of information storage and retrieval must provide for both stability and mutability of the system. A model was proposed allowing for the interplay of both genetic predetermination and experiential modification of neuronal circuits. The basic question of whether the specificity of memory is dependent upon specificity of circuits, macromolecules, or both, cannot be answered at this time. Ironically, proponents of either circuitry or macromolecular specificity can construct reasonable models of responding and recording neural organizations. The main ingredient lacking in such models, however, is adequate data.

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THE ROLE OF WATER STRUCTURES IN THE MOLECULAR ORGANIZATION OF CELL MEMBRANES

Adaptation, dated November 24, 1964,
from a Presentation to the
American Cancer Society Conference on Cryobiology
Rye, New York, October 9-10, 1964

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CONTENTS

* Aided by a fellowship from the Commonwealth Fund and a grant from the Henry Ittleson Family Foundation.

INTRODUCTION

Diverse disciplines and subdisciplines -- each with its own concepts and technics, concentrating on one aspect of membrane function or another -- have contributed to our conceptual picture of cellular membranes in a voluminous literature, difficult to integrate, and impossible to review here. Of the very large number of reviews and monographs dealing with various aspects of biological membranes, we cite three* to give some illustration of the range of problems and experimental approaches in this field. All of the diverse disciplines involved in study of membranes use their own special language and basic assumptions, some of which are unstated. It is apparent that all could converge at the molecular level, making possible a universal language. The goal of molecular membranology should be to formulate a model of cell membranes in a unifying configuration equally satisfactory and meaningful to the biophysicist, physical chemist, biochemist, and physiologist. In this paper I will try to show how, using a new principle of peptide conformation, that it is possible to build a model which provides a unifying configuration for bringing together a variety of ideas, developed by others, dealing with selected aspects of membrane structure and function.

This model may, in particular, be applied to provide a molecular basis for polarization and excitability properties of neuronal membranes.

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MEMBRANE STRUCTURE AND FUNCTION

Structural Observations

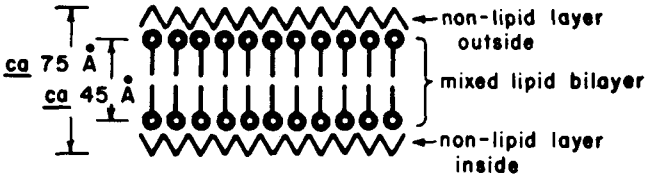
Our concepts of membrane structure were initially derived from physiological studies which attempted to account for two fundamental properties of the cell, ascribed to the permeability barrier designated as the plasma membrane: (a) permeability and active transport processes, and (b) electrical phenomena, e.g. bioelectric potentials, with particular reference to excitability in nerve and muscle.

Studies of a wide variety of cells led to the general conclusion that a relatively thin lipoprotein layer plays a dominant role in the translocation of solutes into and out of the cell. The plasma membrane permeability barrier was studied by a variety of indirect methods, and early speculations concerning its molecular structure were developed by Davson and Danielli (1935; cf. 1952) into their now classical pauci-molecular model of the cell membrane. This model postulated that the cell membrane consists of biomolecular leaflets of lipid, each polar surface of which had on it a monolayer of protein. Later evidence from polarization microscopy, X-ray diffraction studies, and electron microscopy indicated that in several membrane systems the lipid molecules were arranged in bilayers, with the hydrocarbon chains oriented radially (W. J. Schmidt, 1936; Schmitt, Bear, and Clark, 1935; Schmitt, Bear, and Ponder, 1936; Geren, 1954; Robertson, 1955; cf. Schmitt, 1950). Electron microscopy then revealed the characteristic features of a morphological structure at the cell surface, which now is attributed to a lipid-containing membrane of the type predicted from earlier studies. In potassium permanganate-fixed preparations, two narrow dense lines were observed, separated by a band of low density but of similar width, the thickness of the trilaminar unit being about 75 A. (Robertson, 1957). With further study, it became evident that most, if not all, of the characteristic membrane systems of the cell exhibited this trilaminar feature. This morphological uniformity has found expression in the now widely accepted unit-membrane concept, vigorously promulgated by Robertson (1959, 1960, 1962, 1964), which holds that all biological membranes are built on a single fundamental design principle where the basic plan of structural organization consists of two lipid monolayers, sandwiched between two fully spread monolayers

of non-lipid components. There are similarities as well as differences between the present unit membrane concept and the Davson-Danielli model, and these have been discussed by Robertson (1964).

Concurrent with the advent of electron microscopy, technics developed for cell fractionation permitted the isolation of certain characteristic organelles for biochemical studies. Used together, these two technics revealed that cellular membranes exhibit considerable functional diversity as expressed, for example, in profound differences in the types of enzymes bound to different membranes. The association of morphological uniformity with functional diversity in membrane systems may be ascribed in large part to the periodic arrangement of different types of specialized transducing units in a highly ordered lipoprotein matrix, which is fundamentally similar in all membrane systems (cf. Fernandez-Moran, 1957, 1959, 1962, 1964). The transducing units are considered to be ordered macromolecular assemblies, consisting of enzymes and other macromolecules coupled both spatially and energetically, involved in the energized vectorial translocation of ions and other solutes, in contraction, and in other active membrane processes. Though we have some general ideas about the nature of the componentry required for these specialized energized membrane "units" (and an encouraging beginning in approaching this problem has been achieved in mitochondrial membranes where periodically arranged membrane subunits have been visualized (cf. Fernandez-Moran, et al., 1964), neither the chemistry of the essential componentry nor its molecular arrangement in the matrix of the membrane is known with certainty. It is also appreciated that functional differences among membranes may reside in mucopolysaccharides or glycoproteins which may serve as extrinsic coatings of outer membrane layers (cf. Fawcett, 1962). We shall accordingly not attempt to discuss the membrane as a "whole," but concentrate on the lipoprotein matrix which appears to have a structural organization common to most, if not all, cellular membranes.

Figure 1 is a representation of the unit membrane concept. The two non-lipid layers, generally thought to be protein, differ somehow in chemical structure to produce an asymmetrical membrane, and it has been considered that this difference may be related to the presence of glycoprotein or mucopolysaccharides associated with the outer non-lipid layer. Though the thickness of the trilaminar unit membrane is about



Unit membrane concept

Figure 1. A representation of the unit-membrane concept. The asymmetrical nonlipid layers, presumably protein in nature, but of unknown composition and structure, are represented as continuous structures. The mixed lipid bilayer consists primarily of phospholipids; the hydrophilic portion of the lipid molecules are represented by the circles (O) and the extended hydrocarbon chains by the attached tails (|).

75 A, it is recognized that different membranes in the cell may vary in thickness, some being 90-100 A. The lipids in the bimolecular leaflet are a set of phospholipids, mixed with smaller amounts of triglyceride and cholesterol. Variations in membrane thickness can be attributed to differences in lipid constituents and their packing arrangements, to differences in the protein layers, or to specialized transducing units, without fundamental modification of the basic design principle.

A major development in membrane chemistry was made when Green and his associates succeeded in isolating a homogeneous colorless protein from beef heart mitochondrial membrane preparations, which appears to be the protein component of the basic lipoprotein matrix of this membrane system (Criddle et al., 1962; also cf. Green & Fleischer, 1962). Designated as structural protein, it comprises at least 50% of the total protein of mitochondria; and similar structural proteins have been isolated from other membrane systems (Richardson et al., 1963), suggesting that mitochondrial structural protein may be a prototype of the structural protein of membrane systems generally. As isolated, mitochondrial structural protein is a water-insoluble polymer. Following treatment with cationic detergents (or dilute alkali or strong acetic acid), a monomeric species of ca. 25,000 M.W. is obtained which combines with three of the cytochromes (a, b, and c_1) of the mitochondrial electron transfer chain or with mitochondrial lipids to form reproducible complexes. Mitochondrial structrual protein has a higher than average content of non-polar amino acid side chains, and the characteristic tendency of structural protein to polymerize has accordingly been attributed to extensive hydrophobic bonding between monomeric units. The formation of mixed complexes of structural protein and phospholipids and the various cytochromes is likewise believed to be due to hydrophobic bonding predominantly, although other bonding modes are also involved.

Functional Questions

Although some of the chemical details have been filled in, our modern picture of the unit membrane retains many of the fundamental gaps inherent in the original Davson-Danielli model with respect to the physiological role of membranes. Consider, for example, the fact that membranes exhibit high selectivity both for ions and non-electrolytes, some processes apparently involving selective "pores" or "channels"; others, energized transport "pumps." If we consider the membrane as

a heterogeneous structure, where transducing units are arranged in periodic fashion through a lipoprotein matrix, this would account for the energized membrane pump units. The question arises, however, whether the selective pores or channels required for passive transport of solutes are built-in properties of the lipoprotein matrix, or whether specialized devices must be invoked here as well. Our present model of the lipoprotein matrix does not provide an explicit molecular basis for selective pores and channels. We also know that biological membranes, particularly the plasma membrane, exhibit profound changes in state in response to excitation, expressed as nerve depolarization following stimulation and as changes in membrane transport processes induced by certain hormones (e.g. insulin and vasopressin) in responsive cell types. If our present picture of the unit membrane represents one "state" of the membrane matrix, what is the other "state"? More particularly, what is the nature of the phase transitions which must occur in each layer as the membrane changes from one state to another? Following excitation, whether in nerve stimulation or in hormone action, a local perturbation is "somehow" transmitted through the bulk phase of the membrane to influence sites at a distance. What is the molecular basis for the classical problem in physiology designated as "the propagation of a local disturbance"?

Kavanau (1963) has developed a new molecular theory of the membrane structure which provides possible answers to these and other questions about membrane function. Limitations of space do not permit thorough discussion of Kavanau's theory, which gives primary importance to shifts and transitions of the lipid phase of the membrane. Regarding the membrane as a dynamic and labile structure, Kavanau postulates that the membrane shifts between different substructural states with different phases of function, the two extremes being equilibrium states designated as the "open" and "closed" configurations. In the open configuration, it is postulated that the lipids are arranged in cylindrical micelles (pillars) 180-200 A. in thickness, attached at each end to the external protein monolayers 10-15 A. thick, the pillars about 80 A. in width being separated and arranged hexagonally so that there are very large aqueous regions between the pillars. The closed configuration results when the pillars collapse and the lipid micelles either coalesce or closely abut each other, obliterating the aqueous regions. If coalescence between micellar units is complete, this results in a continuous lamina of the familiar bimolecular lipid leaflet;

if coalescence is incomplete, "pores" are formed as the lipid units closely abut. Intermediate states between the open and closed configurations are envisaged; and Kavanau, on the basis of his model, is able to account for a large body of evidence bearing on membrane function in terms of plausible molecular mechanisms.

The lipid phase is the part of the membrane structure we know most about. Ordered arrangements of polar and non-polar lipids in bilayers have been produced and their properties are being studied (cf. Mueller, et al., 1962, 1963; Thompson, 1964). It is reasonable to suggest shifts between ordered lipid bilayers and various micellar arrangements, as demonstrated by Stoeckenius (1962) and Luzatti and Husson (1962), as a plausible basis for phase transition in the lipid layer, when the membrane changes from one state to another, as in the Kavanau theory. If we are to have a complete picture of membrane structure, more information is needed about the molecular organization of the non-lipid components of the membrane, particularly the structural protein of the membrane and the water structures present in the membrane matrix. These two problems are related. It is well-known, in principle, that the aqueous environment is a major determinant of protein conformation, and that the conformation of protein likewise influences the structure of water at the macromolecular surface; changes in one are reflected and find expression in changes in the other. If we but knew the arrangement of water in the membrane, this would help us with our problem of structural protein conformation; contrariwise, if the structure and conformation of the structural protein of the membrane matrix were known, this could serve to help us define the state of membrane water.

Water and Protein Structures

It is now evident from electron microscopy that there are morphologically distinctive types of aqueous regions present in the cell. Water molecules find themselves in different environments, and accordingly different types of water structures may be expected. It is most unlikely that water within the various membranes of the cell, or at the surface of membranes, has the same structure as water in the hyaloplasm. Let us therefore direct our attention to the water structure in the membrane.

It has long been appreciated that water is a bulk component of membrane systems, comprising 30-50% of the total system, and must therefore figure importantly in the molecular organization of the system (cf. Schmitt, Bear and Palmer, 1941; Finean, 1957; Fernandez-Moran, 1957, 1959, 1962, 1964). In an ordered lamellar system, it has seemed reasonable that the water in the membrane must somehow be "highly ordered" in relation to the ordered polar groups of both protein and phospholipids. Fernandez-Moran (1959, 1962) in particular has emphasized the possiblity that water in the membrane may be organized in "ice-like" or crystal hydrate lattices, as an integral structural component of membrane, where it may serve an essential role for various membrane processes.

Localized reversible phase changes in ordered water structure might provide the basis for conformational changes in protein layers and concurrently modify the arrangement of the polar lipids from an ordered bimolecular leaflet to a less tightly packed micellar form. These phase transitions could "spread" through the membrane phase of the cell as water structure changes reversibly, and thus represent the molecular basis for propagation of a local perturbation. Selective permeability might be considered in terms of molecular sieves lined with ordered water; the marked permeability changes induced by excitation could be the consequence of the "melting" of water structures in special regions. By providing an interconnected hydrogen bonding medium, ordered water structures could participate in fast protonic charge transport mechanisms (Eigen and DeMaeyer, 1959), or in electron transport, via hydrogen free radical or hydride ion as suggested by Klotz (1962). The idea of ordered water lattices as an integral structural component of the membrane has great power in providing a conceptual basis for understanding a multiplicity of fundamental mechanisms associated with membrane function.

The difficulty arises when one asks: What is the precise structure of the organized water? Is the ordered water arranged hexagonally as in ice, pentagonally as in clathrate cages, or in yet another form? How many layers of interconnected hydrogen bonded water structures are there, over what distances are they present, and where should they be placed in Fig. 1 to "fill in" our model of the unit membrane? There are no definitive answers to these questions

In the absence of hard evidence it is possible to argue convincingly for either "ice-like" or crystalline-hydrate types of water in the membrane. Though nuclear magnetic spectroscopy offers the potential of a nondestructive method for evaluation of water structures (Fernandez-Moran, 1959), there are profound difficulties in interpreting changes in the proton resonance signal of water in systems of this order of complexity. It has not been possible, as yet, to experimentally approach the arrangement and structure of water in the membrane by looking at water structures directly. May we get at this problem indirectly by considering the structural protein component of the membrane? Though we do not know the conformation or even the amino acid sequence of a single structural protein of a membrane, we may nevertheless ask whether there may not be structural principles from protein chemistry, which might give us insight into the possible conformation of the protein subunits of the membrane, and hence to the water structure.

Caspar and Klug (1962) have expanded the idea of Crick and Watson (1956) that small viruses are built up of idential protein subunits packed together to form a protective shell for the nucleic acid, into a generalized theory for the construction of ordered biological structures, be they virus coats or membranes. The essence of their far-reaching concept is that if one is given as building blocks a large number of identical protein molecules, then it develops that there are only a very limited number of efficient architectural designs for the construction of a biological container: the two basic designs are helical tubes and icosahedral shells. Drawing upon architectural principles inherent in the Buckminster Fuller geodesic dome, wherein steel rods are bonded together in quasi-equivalent triangles grouped in arrays of hexagons and pentagons, Caspar and Klug pointed out how the protein shells of icosahedral viruses and membranes could be assembled from a single type of protein subunit by introducing a suitable ratio of pentamers to hexamers. If the protein matrix of membranes is composed of a single type of protein subunit -- and this appears likely from the studies on mitochondrial structural protein -- the geometrical and architectural principles of the Caspar and Klug theory could be applied to the problem of the protein structure of the membrane.

Robertson (1963, 1964) has considered the Caspar-Klug concept as a possible explanation for his observation by

electron microscopy of a honeycomb-like pattern, involving hexagonal subunits plus a few pentagonal units, in certain favorable frontal sections of synaptic discs and frog retinal rods. The possibility was considered that the observed honeycomb pattern represents a derivative macromolecular pattern localized in the outer surface of unit membranes. From a review of the available literature, Robertson concludes that while a geodesic pattern may eventually be recognized as a general feature of unit membrane systems, further correlative work is necessary, and it seems premature to accept any far-reaching conclusions in this regard.

Despite this cautionary note, let us accept the design principles of Caspar and Klug as a basis for further discussion, and proceed to consider the conformations of a protein subunit which could serve as the equivalents of the hexamers and pentamers required to build a geodesic dome. We know from Caspar and Klug that there are <u>two</u> types of efficient design: helical tubes and icosahedral shells. Does this imply the existence of two fundamental types of protein subunit conformation to serve as the building blocks for these different types of structures? If the α-helical conformation for protein serves for protein structures exhibiting helical symmetry, is there a second conformation of protein utilized for icosahedral symmetry?

When we turn to the subject of protein conformation, I think it is fair to say that here we have one major structural principle: the helix of Pauling. Once, all biological polymers were random; after Pauling's helix, all biopolymers became helical. With increasing experience, it became apparent that only a few proteins are completely helical in structure; most proteins exhibit partial helical structure, the per cent helix varying from protein to protein. Today, it has become clear that the concept of the α-helix, powerful as it has been, does not account for the structure of all proteins (reviewed by Richards, 1963).

Since some proteins do exist in non-helical conformations, one must look to more fundamental unifying principles for protein structure. Such fundamental principles underlying protein structure do arise from energetic and thermodynamic considerations of the folding of peptide chains to achieve a minimal energy state (<u>cf</u>. Caspar, 1963), the folding being dependent on the character and sequence of the amino acid residues of the polypeptide chain, and on the nature of the

solvent environment. In aqueous media, the non-polar groups of a polypeptide have such a high aversion to water molecules that a polypeptide or protein tends to fold in such a way that a maximum number of hydrophobic bonds are shielded from the water by close-packing mutual interactions (primarily via Van der Waals forces), while the interactions of the polar side chains with solvent at the aqueous surface and with each other (as in intrachain hydrogen bonding) secondarily contribute to stability. Depending upon the character of the amino acid sequences involved, the minimal energy state of a protein may be achieved with a helical arrangement, total or partial; but other modes of folding to achieve protein conformation of minimum energy exist. If there is a large preponderance of non-polar amino acid side chains, a minimal energy state may be achieved by polymerization as non-polar regions of the monomer react with each other.

We have already raised the question of whether there may not be a second basic structural principle in peptide and protein conformation, as general and as powerful as the α-helix principle has been, which might be applied not to water-soluble proteins, but to a water-insoluble polymerized system built of hexamers plus a few pentamers. Dr. Donald Warner in a series of theoretical publications (Warner, 1961a, 1961b, 1964) has developed a new approach to peptide and protein conformation, which we may call the "hexagonal concept." This hexagonal conformation is of interest because it permits us to visualize how hexamers might be packed with a few pentamers and with water to form the protein coats of membranes. Additional principles, inherent in the hexagonal conformation emerge which have striking implications for membrane function as well as structure.

HEXAGONAL CONFORMATION MODEL FOR PROTEINS AND PEPTIDES

Warner undertook the study of molecular models of several peptide hormones and peptide antibiotics to determine whether it might be possible to discover a unifying structural feature or arrangement of peptide bonds in biologically active peptides. Using molecular models, Warner found that the peptide bonds of various polypeptides studied could be arranged to form regular hexagonal patterns in which all of the carbonyl oxygens of the peptide bonds occupy positions on one planar surface, the oxygens being so arranged that they form the corners of a hexagonal unit, and all the amino acid side

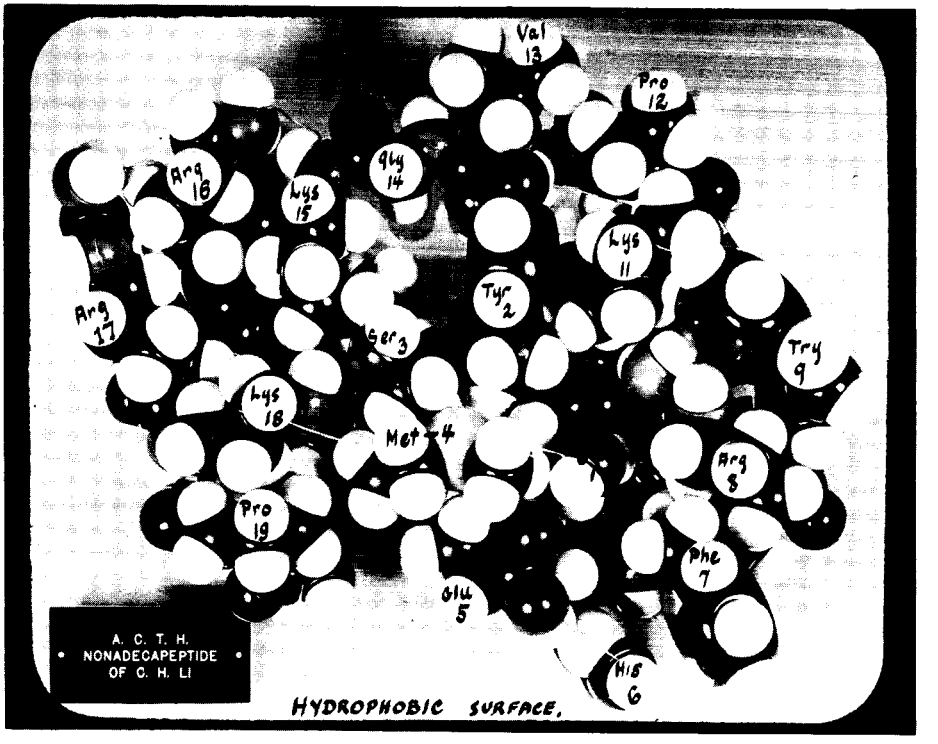
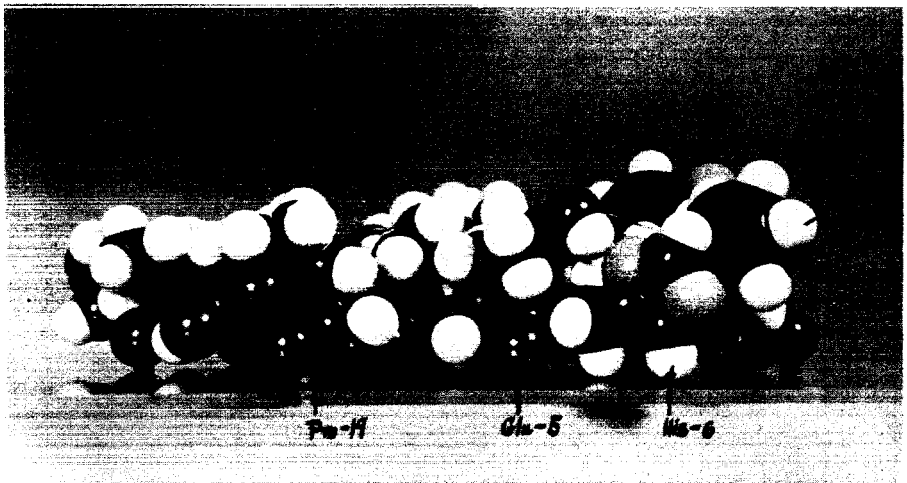
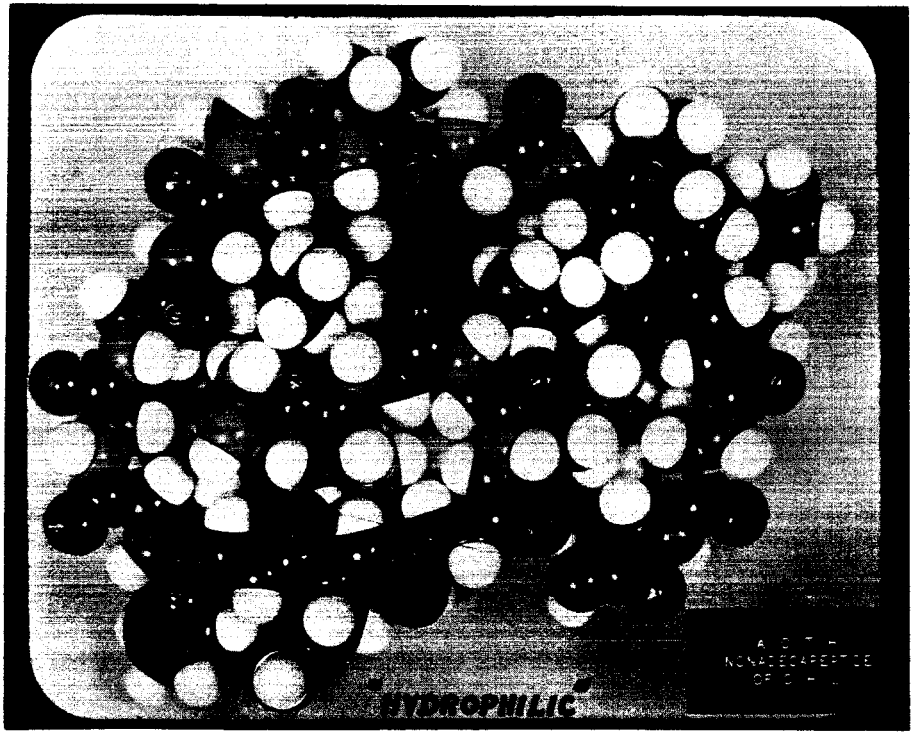


Figure 2. The model of the nonadecapeptide of ACTH of Li constructed on the basis of the hexagonal concept of Warner, showing the hydrophobic, edge, and hydrophilic views. This model differs in several respects from a previous Warner model of this molecule shown by Li (1962). The amino acid residues are numbered successively from the N-terminus. Black: carbon, grey: oxygen, white: hydrogen.



Edge View
"1-14" ACTH



chains project downward to form the second surface of the model. This type of hexagonal arrangement gives rise to a planar face, designated as "hydrophilic," containing the carbonyl oxygens of the peptide bonds; the second surface produced by the closely packed side chains being designated as "hydrophobic." It was found in the models of small peptides studied, that the polar amino acid side chains of the hydrophobic face frequently were so placed that stabilizing hydrogen bonds between neighboring polar groups could be easily envisaged, and that hydrophobic interactions of the non-polar side chains were also likely contributing to conformational stability. Warner called attention to the fact that the 4.8 A. distance (oxygen center to oxygen center) in the carbonyl oxygen pattern of the hydrophilic face of these hexagonal peptides coincides with the "second-neighbor" oxygen pattern of water in a hexagonal arrangement, and he visualized hydrogen bonding between hexagonal water units and the hydrophilic surface of the peptide. Using these principles, Warner built a series of models of linear peptide hormones of increasing length: the N-terminal decapeptide of ACTH (10 amino acids), the N-terminal nonadecapeptide of ACTH (19 amino acids), and B-chain of insulin (30 amino acids). He then extended the hexagonal concept to the protein subunit of the tobacco mosaic virus and considered how subunits might be arranged to construct a protein coat for the virus (Warner, 1964).

To illustrate the hexagonal principle, let us examine the Warner model of the N-terminal nonadecapeptide ACTH of Li (1962). The hydrophilic and hydrophobic surfaces and an edge view of the model are shown in Figure 2. I have elsewhere (Hechter, 1965) considered the hexagonal conformation of hormonal peptides in relation to the recognition phenomenon in the interaction of peptide hormones with specific receptors in the membrane. In this connection, the question arose whether the monomeric form of the Warner model of the ACTH nonadecapeptide on energetic grounds could be considered as a likely conformation at the receptor site in the responsive cell. The answer was yes, provided that one assumed that the receptor site contained a flexible peptide component, complementary to the hydrophobic face of the peptide hormone. Utilizing and extending Koshland's concept (1962), it was not difficult to envisage how in the presence of hormone, <u>both</u> receptor and peptide might undergo successive configurational changes as one component interacts, group by group, with the complementary component to produce a two-disc system (interlocked through hydrophobic faces). In such a system, all the

non-polar side chains (except for edge groups) would be buried in the center; the groups exposed to water would be the polar carbonyl oxygens and α-NH groups of the peptide bonds. This is an interesting solution to the energetic problem: two peptide configurations, inherently unstable when the units are isolated, in effect become a stable system when the units are locked together, through the collective effect of a number of weak forces.

With this background, the question may now be posed whether the fundamental protein matrix of the membrane involves the packing of identical hexagonal subunits in a regular manner together with some pentagonal units. In this connection Warner's (1964) application of the hexagonal principle to the protein coat of the TMV virus assumes special interest. Warner postulated a hexagonal conformation for the protein subunit (whose amino acid sequence is known), and then proceeded to consider on theoretical grounds how the hexagonal subunit discs must be packed to form a protein coat for the TMV virus, and conform to the available physical data bearing on this problem. It should be noted that the subunit conformation and packing arrangement suggested by Warner for the protein coat of the TMV virus are very different from the generally accepted model (cf. Caspar, 1963) which assumes a helical conformation of the protein subunits packed in a helical array. Important as this point may be, the question of which conformation for the TMV virus is to be preferred does not concern us here. The important point for this discussion is that in the conceptual design utilized by Warner for locking hexagonal disc subunits together, certain principles will emerge which I feel may be of fundamental interest to the possible elucidation of certain key problems of membrane function.

Let us therefore re-examine Warner's studies on the protein of the TMV virus from the point of view of membrane organization, independent of whether or not it applies in the TMV case. Figure 3a shows Warner's schematic arrangement of 6 peptide subunits locked together through hydrophobic surfaces to form a hexameric A-protein unit, it then being postulated that these A-protein units are "cemented" together at the hydrophilic surface by two water layers to form the protein coat illustrated in Figure 3b. Two fundamental assumptions were here made by Warner: (a) that the distance between the hydrophilic surfaces of the two peptide subunits interlocked at the hydrophobic surfaces is about 6.9 A. (from

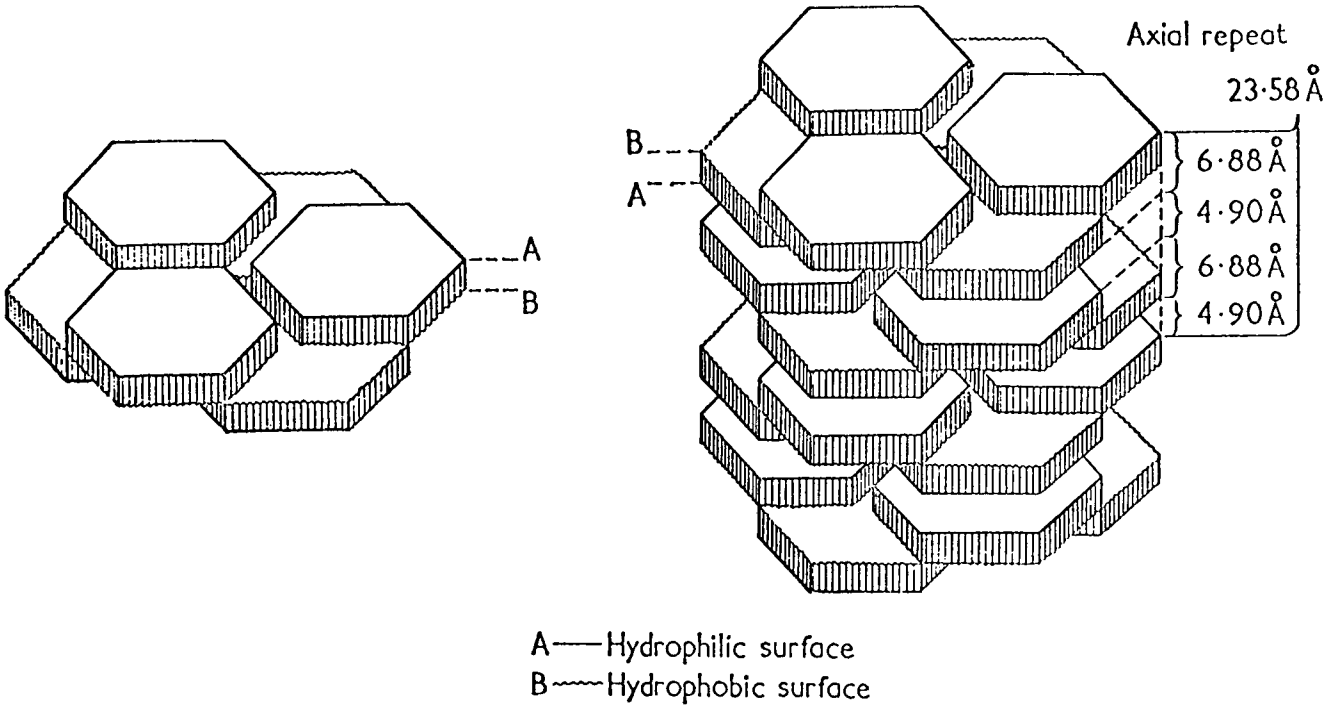


Figure 3. The principles used by Warner to pack hexagonal protein subunits of the TMV virus are shown.

Figure 3a is a schematic A-protein unit composed of six protein subunits, arranged three on three, via interactions of their hydrophobic surfaces.

Figure 3b is a schematic to illustrate how a protein coat for viral RNA might be formed by axial layering of the hexameric A-protein units.

center to center of the carbonyl oxygens of the two surfaces); (b) that the hydrophilic surfaces of adjacent A-protein units will be separated by a space of 4.9 A. containing two "second-neighbor" water oxygen layers. In effect Warner postulated that the two water layers which play a structural role in his model are highly ordered and arranged hexagonally in an ice-like lattice. It is apparent that this idea has significance for our previous discussion of the state of water in membrane systems.

The overlap pattern which results from the 6-subunit arrangement of Warner's model of the A-protein unit (Figure 3a) is shown in Figure 4. It will be seen that a central "hole" or "channel" arises where the individual hexagonal discs do not overlap. The amino acid residues at the edges of this hole comprise a total of 12 carboxyl groups* and 24 hydroxyl groups which are quite uniformly distributed around the edge; there are no cationic side chains (arginyl, lysyl, or histidyl) at the edge of this hole. Warner's arrangement gives rise to a channel lined with carboxyl groups, some of which are COO^-; and the hydroxy groups at the edge represent hydrogen-bonding sites available to water. In effect, the hexameric arrangement of Warner of three on three creates an aqueous channel lined with hydrogen bonded water, possessing COO^- sites for binding cations. Such a channel has obvious implications for the problem of ion selectivity in biological membranes, since it is permselective for cations and would exclude anions. Depending upon the specific dimensions of the channel and the nature of the amino acid residues at the edge, the possibility of selectivity between cations can also be envisaged. As one continues to use hexagons to fill up the two-layered disc system, new channels emerge, where the side chains at the edge comprise hydroxyl groups and basic groups of amino acids (primarily arginine) to produce an aqueous channel permselective for anions.

The suggestive implications for membrane structure and function that arise out of Warner's design for a protein coat of the TMV virus may represent trivial coincidences. I do not think this is the case. Warner's concept, suitably

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* Some of these 12 carboxyl groups may be replaced by carboxyamide groups if the slight modifications of the TMV sequence proposed by Anderer, E. F. and Handschuh, D. (Naturforsch 17b, 336, 1962) are verified in future work.

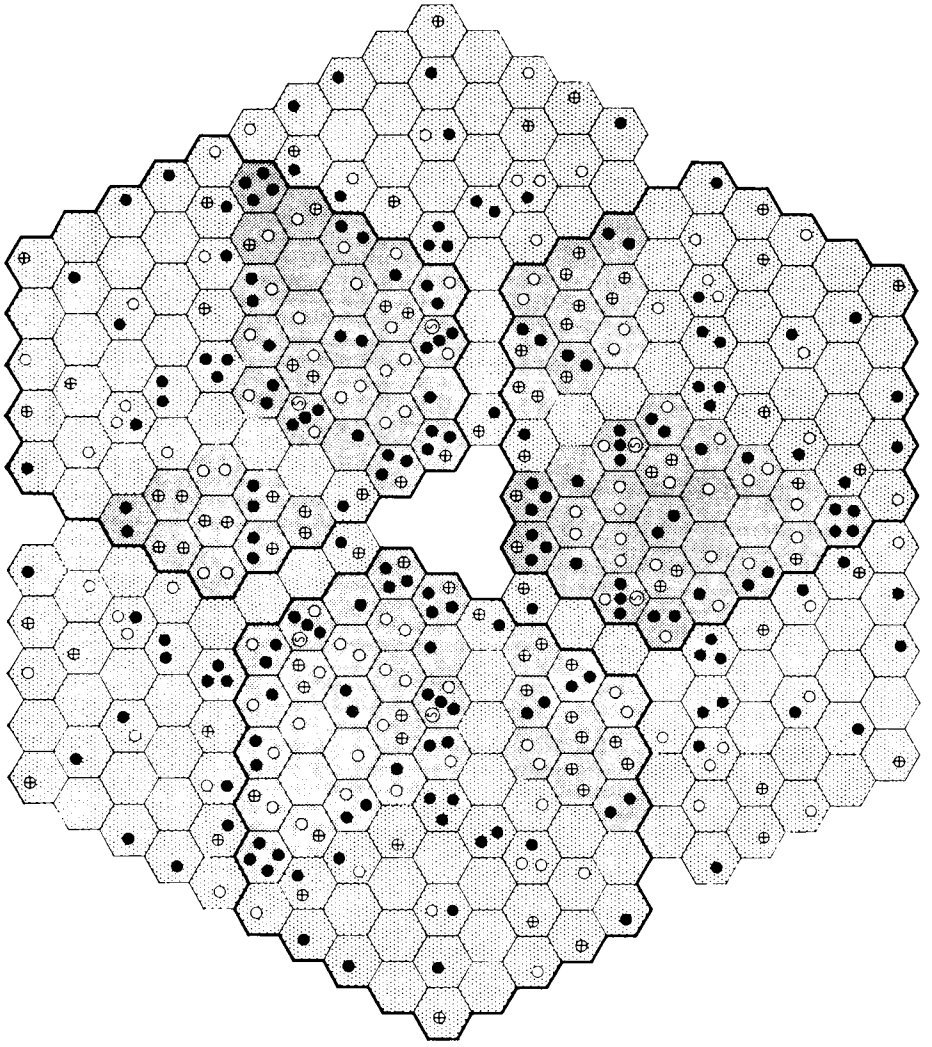


Figure 4. The overlap pattern achieved in the A-protein unit (Fig. 3a). The three upper subunits are outlined in black; ●, aliphatic hydroxyl side chains; ⊕, carboxyl side chain; ○, amide side chain; S, sulfhydryl group.

translated, provides a basis for a fresh look at membrane structure in terms of protein subunits arranged primarily as hexamers, with a few pentamers. These ideas may or may not apply to the proteins of the membrane in its totality with enzymes, "pumps," contractile elements, and other specialized transduction devices, but they may illustrate the basic molecular organization of the protein envelopes of the membrane, and help us to approach certain aspects of membrane function.

HEXAGONAL-SUBUNIT MODEL FOR CELL MEMBRANES

Let us accept the basic molecular features of the unit membrane thesis as a basis for discussion and fill in the protein layers primarily with hexagonal peptide subunits locked together as hexamers as in the TMV model of Warner. Important as the problem of extrinsic coatings of mucopolysaccharides or glycoproteins at the outer membrane surface may be for the arrangement of water molecules at the surface, in the absence of definitive chemical evidence this aspect of the problem will not be here considered. Using this model, let us now consider the nature of membrane changes associated with the classical problem of depolarization in nerve.

Figure 5 is a representation of a model of a membrane region in resting state, relatively impermeable to sodium ion, but not to potassium. The protein phase of the membrane model is built of two layers of peptide-antipeptide units, each interlocked disc system being 6.9 A. in thickness, separated by two water layers in a hexagonal ice-like arrangement, hydrogen-bonded via second-neighbor relations to the carbonyl oxygens of the peptide hydrophilic surface. In this arrangement the outer protein layer is about 19 A. thick, which is about the right order of magnitude for a plasma membrane of 85-100 A. If one wished to decrease the thickness of the protein layer, removal of one layer of interlocked hexameric discs would reduce the protein layer to 6.9 instead of 18.7 A.

From the overlap design pattern in the TMV model of Warner, we now have "channels" edged with hydroxyle groups and either positively or negatively charged side chains. These charged aqueous channels are filled with ordered water, some water layers being tightly hydrogen-bonded to the edges, other water molecules being more mobile but still restricted in mobility; the effective dimensions of the channel would

depend upon the number and arrangement of the mobile water molecules in the water channel. In such channels selectivity between cations would depend upon the size and shape of the hydrated ion. Since the potassium ion has only one or two water molecules in its immediate hydration shell, whereas sodium has at least six, K^+ is a smaller hydrated ion but also has a different shape. It is not difficult to see how a cationic channel of this type might be selectively available for potassium but not for sodium ion. We will arrange the cationic and anionic channels of the locked subunits of each hexameric layer in staggered relation, so that the cationic channel of the first set is above an anionic channel in the next layer.

The protein layer in turn is associated with a conventional mixed lipid bilayer via two water layers in an ordered ice-like arrangement. Warner and I have found that the carbonyl oxygens of the ester bonds of triglyceride can be fitted perfectly into the hexagonal water structure, these oxygens replacing the oxygen in water. It would be necessary to arrange the polar groups of the phospholipids so that they likewise fit the ordered water layers. Warner and I have studied structural models of several typical phospholipids, and there appear to be no special difficulties in arranging the polar groups in this fashion. The lipid layer illustrated shows extended hydrocarbon chains partially interdigitated, but it is of course possible to pack the hydrocarbon chains more or less tightly. Finally, we come to the innermost protein layer of the membrane, which we know in principle must be different than the outermost protein layer in important respects, but about which we know very little indeed. The required asymmetry might well be related to extrinsic coatings of polysaccharide at the outer surface, but there are other possibilities, e.g. a different type of subunit structure or protein organization in the inner face of the membrane, etc. The question of asymmetry need not concern us at this juncture; so for purposes of illustration, we will consider the inner protein layer of the lipoprotein matrix to likewise consist of locked hexagonal units.

In effect we have drawn in Figure 5 a lattice arrangement whose ordered stability depends on a set of interlocking weak forces acting in concert. The arrangement of water in a hexagonal ice-like structure between protein layers depends upon a variety of weak forces (hydrophobic and hydrogen bonding) locking the hexagonal subunits together, and the

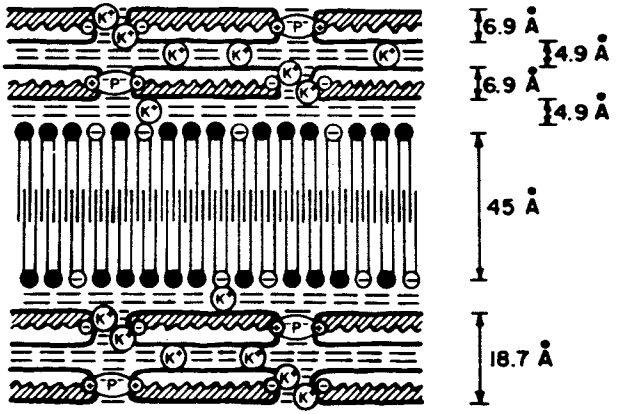


Figure 5. A schematic representation of the "resting membrane," where the basic features of the unit-membrane concept are retained and the protein layers are represented as a system of interlocked hexagonal discs "cemented" together by water layers in an ice-like arrangement to form a precisely ordered lattice system. The individual hexagonal subunits are shown as "interlocked" through hydrophobic surfaces to form "disc units" held together by two layers of water in an ice-like state, this type of water being represented as (————). The aqueous channels in the protein layer of the membrane possess fixed charge sites, and are shown in a "staggered" relationship; most of the water in the aqueous channels has an "ice-like" structure. The bilayer arrangement of the mixed lipids, involving interdigitation of fatty acid tails, is assumed to be dependent upon the "ice-like" layers of water, which fix the hydrophilic portion of lipid molecules in position in relation to the hydrophilic surfaces of the neighboring protein subunits. Potassium is shown as the principal counterion for fixed negative sites in the resting membrane, phosphate as the counterion for fixed positive sites; but other ions possibly involved are not shown. In this model, selectivity for potassium over sodium ion depends upon the organization of the membrane units to form a precise lattice, as discussed in the text.

conformation of the locked peptide subunits depends in part upon the water structure. Perhaps at some points the interlocked hexagonal units are crosslinked by disulfide covalent bonds for added structural stability. The ordered lipid bilayer depends in turn upon the hexagonal ice-like water structure in alignment with the hydrophilic surfaces of peptide subunits; and this in turn influences the arrangement of the inner protein layer. It is apparent that the staggering of the fixed charges on the channels and the ordered structure of most of the water at hydrophilic surfaces produce considerable restrictions to the free diffusion of water-soluble permeant species. Our maze-like arrangement of charges in effect constitutes a formidable barrier to the translocation of any charged species, be they cations or anions. Furthermore, it is not difficult to visualize selective translocation of sugars and other non-electrolytes in a system of this type.

Assuming appropriate dimensions, it is now possible to envisage how potassium ions are available to act selectively as the principal counterions at the fixed negative sites in the protein channels. Divalent ions, perhaps Mg^{++} or Ca^{++}, may serve to lock negative sites together at critical points. The fixed positive sites likewise require a counterion, perhaps phosphate or chloride. The hydrated sodium ion would be too large to penetrate the cationic aqueous channels pictured, containing highly ordered water or the hexagonal ice-like layers cementing the subunits together. Such an ordered lattice system thus resembles a potassium electrode, where the permeability of potassium is low, but where conductance is high. It is apparent that the schematic model of Figure 5 is a possible molecular representation of the long-pore theory, which attempts to explain how a membrane with low potassium permeability nevertheless exhibits high conductance (cf. Lea, 1963); it also is a representation of the classic molecular sieve theory of Boyle and Conway (1941) where ion selectivity in passive permeability processes was related to the relative dimensions of "pores" and hydrated ions, but takes "edge-effects" into account.

Let us now disturb the system at a local point near the outmost surface of the protein layer. At this point in our discussion we are not concerned with the precise mechanism of how the local perturbation is initiated, be it by (a) the opening of a strategic S-S bond which bridges two hexagonal subunits together, by the injection of electrons into the system so that the reaction $S\text{-}S + 2e^- + 2H^+ \longrightarrow 2SH$

is enabled to proceed, or (b) the introduction of a molecular species which either disturbs the complementary interactions of paired hexagonal units so that a conformational change occurs or modifies the water structure of an aqueous channel. In all cases the initiating excitation produces a local change in the associated water structures. If, as we have assumed, the ice-like arrangement of the water layers contributes to the conformational stability of the locked neighboring hexagonal peptide units, we may expect that as water structures are changed locally, configurational changes occur in neighboring hexagonal units, so that in effect a local perturbation in water structures is enabled to spread through the outer protein layers of the membrane, and also involve the ordered water layers which maintain the lipid phase as a bimolecular leaflet, eventually influencing the inner protein layer.

Figure 6 is a schematic representation of a depolarized membrane, where the conformation change in the hexagonal protein subunits is shown as a change in molecular configuration toward a more globular form, perhaps as a tendency for development of a partial helix, and where the lipid phase tends toward a micellar arrangement (for purposes of illustration, extremes of configuration change are depicted). It is apparent that in this state, the precise ordered lattice arrangements in the resting membrane no longer apply. The water in the aqueous channels is less organized in relation to the peptide surfaces, more mobile and increasingly available to serve as solvent for ionic diffusion. Although there are still fixed charged sites, their arrangement is no longer precise, and the modified water serves to create new channels so that sodium and potassium can diffuse rather freely into and out of the cell, with the electrochemical gradient. Our representation implies that all "pieces" of the membrane must fit together if the precise lattice is to be maintained, and that a localized change in a single component, which may be minor, produces widespread changes in the other components, disturbing the arrangement of the system as a whole, so that it no longer is a precisely-ordered lattic.

We have already implied that if non-polar groups, whether from peptide or steroid hormones or other biological activators of cell function, were fitted into appropriate sites of the aqueous channels, the hydrocarbon character of these groups would tend to disturb local water structure. Whether a collection of appropriate non-polar groups could serve to

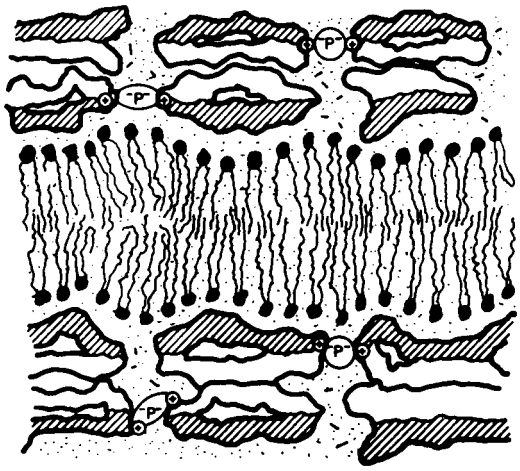
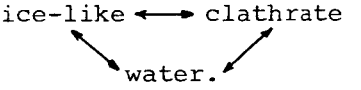


Figure 6. A schematic representation of the "depolarized" membrane, where the arrangement of protein subunits, lipids, and of water no longer provide a precisely ordered lattice. For purposes of illustration, the hypothetical changes in the various structural components of the membrane are highly exaggerated. The protein subunits have changed from hexagonal discs to a more globular helical form, the lipid bilayer to a more random micellar arrangement, and the "ice-like" water structures to less ordered water structures. In consequence, the fixed negative sites in the depolarized region of the membrane no longer exhibit high selectivity for potassium over sodium. Mobile water molecules, represented as small dots (⋯), thus become available and provide aqueous channels which permit relatively free diffusion of cations with the electrochemical gradient. The phosphate cross-links between neighboring fixed positive sites are shown as undisturbed, to indicate that the membrane structure does not break down completely; other links which maintain membrane structure and which provide the basis for reversal to the "resting" membrane state are not illustrated.

create "cages" of water clathrates as Klotz (1960) has suggested, or other modes of disturbance in the local water structure are considered (cf. Kauzman, 1959; Scheraga, 1961), such effects could be widespread through the type of membrane system suggested here. These considerations take on special significance in relation to the hydrate microcrystal theory of anesthesia advanced by Pauling (1961) and Miller (1961). To explain the anesthetic effects of rare gases like xenon, argon and non-hydrogen-bonding anesthetics, the concept was developed that these agents act in membrane systems by forming stable clathrate cages of water (pentagonal, dodecahedral, etc.) which interfere with nerve conduction.

Richards (1963) has pointed out that the formation of complete clathrate cages around a non-polar group at a planar or convex surface of a protein in aqueous solution is difficult to envisage in more than two-dimensional order -- that the stability of clathrates is dependent upon extended order in three dimensions. In the aqueous regions of the membrane model we have presented, limited to a few water layers, the stability of two-dimensional lattices around the non-polar side chains of proteins may be markedly enhanced, there being no bulk solvent to bombard the clathrate cage. The possibility emerges as an attractive idea that the suggested propagation mechanism of changing water structures in the membrane involves the formation and breaking of clathrates, as peptide units undergo conformational change and present non-polar groups to aqueous regions. Phase trasitions in membrane water would then involve the following shifts:



Note Added, Nov. 24: Person and Zipper (1964) recently reported experiments in which mitochondria were disrupted, and the cytochrome oxidase complex solubilized, by treating mitochondrial suspensions with a dry synthetic zeolite. The authors present evidence that this disruption is dependent in large part upon the avidity of the zeolite for the water of the mitochondrial membranes. The authors also call attention to Hanahan's suggestion (1960) that the lipid and protein moieties of ordered lipoproteins are held together by water structures.

CONCLUSION

This presentation has considered the structure of water in relation to the problem of molecular organization of biological membranes. It was suggested that the solution of the problem of organized water in the membrane was inextricably linked to the conformations of the structural protein of the fundamental lipoprotein matrix of the membrane. The usefulness of a new structural concept of protein and peptide conformation, adequate to deal with water-insoluble structural proteins and to form hexamers (and pentamers) arranged in geodesic patterns, was suggested. The hexagonal concept of Warner, an idea in peptide and protein structure developed primarily on chemical structural grounds, was then examined as a possible basis for forming the protein coat of a membrane and for approaching certain problems of membrane structure and function.

The conceptual configuration presented about membrane structure may be incorrect with regard to specific detail, perhaps in basic principle. But I should emphasize that if we are ever to understand the membrane at a molecular level in terms of both structure and function, we must clearly recognize that our primary difficulty may be the absence of a holistic concept sufficiently powerful to bridge the individual findings and atomistic concepts of the diverse disciplines involved in this problem.

ACKNOWLEDGMENTS

The model of the cell membrane presented was initially developed as a consequence of attending a Work Session of the Neurosciences Research Program dealing with membrane structure and function,* on the basis of stimulating discussions with Drs. H. Fernandez-Moran, J. D. Robertson, A. Katchalsky, D. E. Green, and F. O. Schmitt. Later discussions, particularly with Drs. D. T. Warner and H. Fernandez-Moran, served to clarify many of the issues involved; their special assistance is gratefully acknowledged. If the concept presented here should prove to have merit, in very large part this is due to the criticism and encouragement received from all of these individuals; the author retains exclusive rights to all of the inadequacies and deficiencies of the model presented.

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* Summarized by the Work Session Chairman, Dr. A. L. Lehninger, in NRP Bulletin, Vol. II, No. 2, March-April, 1964.

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N E W S A N D V I E W S

On Meetings and Organizations

Dec. 7-11, Closed; only open meeting, Dec. 9 — Symposium on Cell Membrane Problems, Buffalo, New York (Mrs. Muniak, Organizing Secretary, 210 Winspear Ave., Buffalo, N. Y., 14215)

Dec. 26-31 — American Association for the Advancement of Science (Annual), Montreal, Canada. (R.L. Taylor, AAAS, 1515 Massachusetts Ave., N.W., Washington, D.C. 20005)

1965

Jan. 6-9 — Psychopharmacological Conference, Czechoslovak Med. Soc., Psychiatry Section, Jesenik Spa.
(M. Vojtechovsky, Budejovicta 800, Pavilion A1, Prague, Czechoslovakia)

Sept. 1-9 — International Congress of Physiological Sciences (23rd) Tokyo, Japan.
Travel grant application due: 1 January 1965
Write: USA National Committee, International Union of Physiological Sciences, Room 256, 2101 Constitution Ave., N.W., Washington, D.C., 20418

Sept. 5-10 — Eighth International Congress of Neurology, Vienna, Austria.
The three main topices of the Congress are: Late Sequels of Head Injury, Dr. E. Herman, of Lodz, Chairman; Neuromuscular Diseases, Dr. R. Carcin, Paris, Chairman; and Disturbances of the Occipital Lobe, Dr. H. Hoff, Vienna, Chairman. Topics 2 and 3 are scheduled as joint symposia with the Sixth International Congress of Electroencephalography and Clinical Neurophysiology.
The deadline for the submission of free communications is Dec. 30, 1964. A 500-word summary with the full manuscript of the presentation are due before May 15, 1965.
Registration fee is $30.
Inquiries regarding the Congress should be addressed to the Secretary General of the Congress, Dr. H. Tschabitscher, Weiner Medizinische Akademie, Alserstrasse 4, Wien IX, Austria.

About People

Studies of Macromolecular Biosynthesis, edited by Associate RICHARD B. ROBERTS, has recently been published by the Carnegie Institution of Washington.

Cornell University has named ROBERT S. MORISON director of the newly established division of basic biology. He has been director of Medical & Natural Sciences for the Rockefeller Foundation since 1959.

Newly appointed Hill Professor of Neuropharmacology at the University of Minnesota Medical School is AMEDEO S. MARRAZZI, Director of the Veterans Administration Research Laboratories in Neuropsychiatry and Professor of Physiology and Pharmacology at the University of Pittsburgh School of Medicine.

Serving for a year as a visiting professor at the University of Göttingen, West Germany, is LOUIS L. TUREEN, Professor of Clinical Neurology and Psychology, and Chairman of the Section of Neurology at the St. Louis University Medical School.

A grant is to be made by the Wellcome Trust in London to DR. V. C. ABRAHAMS and DR. E. P. LANGWORTH, Queen's University, Kingston, Ontario to establish the necessary computer and tape system for their joint research with other university departments in a program of research on communication theory, particularly on organization in the brain stem.

Programs of Interest

The Brain Research Foundation, Inc., of Chicago, has formally affiliated with the University of Chicago in a joint program for the advancement of research and education on the brain and nervous system. George W. Beadle, President of the University, and William E. Fay, Jr., President of the Foundation, said in their joint statement announcing the plan, "We believe this collaborative program will strengthen the University's ongoing work in research, teaching, and patient care in this field. We also believe it will strengthen the Foundation's ability to foster and encourage research and education on problems of the brain".

Fellowships: NSF has opened six fellowship programs for the support of scientific study and research. These include: Graduate Fellowships, Cooperative Graduate Fellowships, Postdoctoral Fellowships, Science Faculty Fellowships, Senior Postdoctoral Fellowships, and NATO Postdoctoral Fellowships in Science. For further information on these programs write to the National Science Foundation, Washington, D.C., 20550.

From Publications

The October 1964 issue of Bioscience contains an article "Some Contributions of Exotic Animals to Biomedical Research" in which its author, Charles G. Wilber, writes:

> "One wonders whether the electrophysiologist could survive without the giant axon of the squid... In this species, there is a giant axon which is easy to dissect out and which lends itself readily to experimental manipulation... It has contributed much to our knowledge of ion shifts, the mechanism of polarization, mechanism of impulse transmission, speed, amplitude, and environmental effects associated with nervous function.
>
> "The crab has also played an important role in the study of nerve physiology... It can be said with truth that 'most of the available information on heat production stems from the experiments on the frog or crab nerve.'
>
> "The experiments which have been so neatly and carefully done on these lowly exotic animals have been confirmed in a few instances using mammalian nerves, which are intrinsically difficult to study." [Editor's underline]

William Goffman and Vaun A. Newill, of Western Reserve University, have written on "Generalization of Epidemic Theory; an Application to the Transmission of Ideas" in the October 17, 1964 issue of Nature. The authors suggest:

> "Since the process of transmitting ideas, as in the case of an infectious disease, is not

> a single process within a population but a collection of interacting processes within sub-populations, it would seem that the notion of an all-encompassing information retrieval system spanning the totality of knowledge should be replaced by the notion of small dynamic interrelated systems that appear when needed and disappear when not needed."

"Information Processing in Computer and Man" was discussed by Herbert Simon and Allen Newell in the September 1964 issue of American Scientist. Their view of the applicability of information-processing theory to neurophysiology was expressed as follows:

> "Although none of the advances that have been described constitute explanations of human thought at the still more microscopic, physiological level, they open opportunities for new research strategies in physiological psychology. As the information-processing theories become more powerful and better validated, they disclose to the physiological psychologist the fundamental mechanisms and processes that he needs to explain. He need no longer face the task of building the whole long bridge from microscopic neurological and molecular structures to gross human behavior, but can instead concentrate on bridging the much shorter gap from physiology to elementary information processes."

Regarding macromolecular coding of information, the authors said:

> "Evidence as to how information is symbolized in the brain is almost nonexistent. If the reader is assisted by thinking of different symbols as different macromolecules, this metaphor is as good as any. A few physiologists think it may even be the correct explanation. See Holger Hyden, 'Biochemical Aspects of Brain Activity,' in Farber, S. M. and Wilson, R. H. L. (eds.), Control of the Mind (New

> York: McGraw-Hill, 1961), pp. 18-39. Differing patterns of neural activity will do as well. See Adey, W. R., Kado, R. T., Didio, J., and Schindler, W. J., 'Impedance Changes in Cerebral Tissue Accompanying a Learned Discriminative Performance in the Cat,' Experimental Physiology, 7, 259-281 (1963)."

John R. Platt, writing on "Strong Inference" in the October 16, 1964 issue of Science, suggested that progress in molecular biology has been due in large part, to the systematic application of inductive inference. As an example of the widespread use of this approach Dr. Platt said:

> "Start with the first paper in the Journal of Molecular Biology for 1964 (9), and you immediately find: 'Our conclusions... might be invalid if ... (i) ... (ii) ... or (iii) ... We shall describe experiments which eliminate these alternatives.' The average physicist or chemist or scientist in any field accustomed to less closely reasoned articles and less sharply stated inferences will find it a salutary experience to dip into that journal almost at random."

The paper cited, "Interruptions in the Polynucleotide Strands in Bacteriophage DNA," was written by P. F. Davison, D. Freifelder and B. Holloway.

The October 30, 1964 issue of Science contains a long letter by Kent Chernetski, describing his two-month post-doctoral visit to Y. Katsuki's laboratory in the Department of Physiology, Tokyo Medical and Dental University. The U.S.-Japan Cooperative Science Program, which sponsored Dr. Chernetski's visit, also sponsored the Symposium on Neurophysiology (March 5-10, 1964) reported by Theodore Bullock in the May-June 1964 NRP Bulletin.

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